Conservation Genetics of the World's Most Endangered Seabird, the Chatham Island Tāiko *(Pterodroma magentae)*

Hokopapa o tch Tchāik
Whakapapa o te Tāiko

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Ko te manu e kai ana i te miro, nōna te ngahere

Ko te manu e kai ana i te mātauranga, nōna te ao

The bird that partakes of the miro berry has the forest,

The bird that partakes of knowledge has the world

The rākau momori (tree carving) above is the cultural and intellectual taonga of the Moriori people and has been reproduced with the permission of Hokotehi Moriori Trust.

Abstract

The research field of genetics provides useful tools to investigate the biology of species that are difficult to observe and study and are especially valuable in guiding the conservation of endangered species. The Chatham Island Tāiko (Tchāik, *Pterodroma magentae)* is the world's most endangered seabird with an estimated population size of just 120-150 birds, including only 8-15 breeding pairs. This thesis used genetic techniques to investigate aspects of Tāiko biology and relationships in order to aid Tāiko conservation. The mitochondrial cytochrome *b* gene and duplicated regions of domain I of the mitochondrial control region were DNA sequenced in almost the entire known Tāiko population. The level of genetic variation revealed in Tāiko was unexpectedly high considering endangered species typically exhibit low genetic diversity. Sequencing of ancient DNA from subfossil Tāiko bones allowed an investigation of the past level of genetic variation and the species' previous geographic distribution. A large proportion of the genetic diversity of the extinct Tāiko populations was retained in the remnant population. However, genetic variation in Tāiko chicks was low, thus genetic diversity in the population could be lost in just a few generations. There are many non-breeding Tāiko so DNA sexing was used to examine sex ratios in the population. Almost all unpaired birds were male, which signified a potential Allee effect (i.e. that a reduced density of potential mates is decreasing population productivity). Further understanding of the Tāiko mating system and behaviour was obtained by parentage, sibship and pairwise relatedness analyses of genotypes at eight microsatellite DNA loci. It is important that Tāiko are found so they can be protected from introduced predators. The results of mitochondrial DNA sequencing and microsatellite DNA genotyping indicated that there are likely to be more Tāiko breeding in undiscovered areas. Analysis of philopatry using both mitochondrial and nuclear markers can assist conservation by the identification of areas to search for these undiscovered individuals. Tāiko may have once and could still be found on islands near South America since DNA sequencing showed the Magenta Petrel type specimen (collected in 1867 in the South Pacific Ocean) is a Tāiko.

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Ehara taku toa i te toa takitahi engari he toa takitini
Not the strength of one alone, but that of many

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Thesis Structure, Financial Support and Regulatory Compliance

This thesis begins with a general introduction (chapter one) providing the background and intellectual framework that underpins the thesis. Details of the focal species, its cultural importance, history and conservation have also been included. Further chapters two to seven have been written as 'stand-alone' scientific papers. Therefore some information provided in the introduction will be briefly outlined again in chapter introductions. The final chapter (eight) is a discussion of the conclusions and applications of the research findings and potential future research.

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Ethics Approval

This research had ethics approval from the New Zealand Department of Conservation (DOC) Animal Ethics Committee for sampling from Tāiko (AEC 43). After consultation with imi / iwi and the Chatham Island Conservation Board, the Institutional Biological Safety Committee of Massey University granted permission for cloning (GMO 03/MU/15). The collection of Tāiko bones from natural deposits was covered by DOC permit no. WE/116/Res. I also obtained permission for the collection of Tāiko bones from the Chatham Island Conservation Board, landowners, the Hokotehi Moriori Trust, Te Runanga o Wharekauri Rēkohu and some members of Ngāti Mutunga. All bones once sampled were returned to the Chatham Islands within two years of collection, as agreed.

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Chapter One

General Introduction

The biology and ecology of a species can be investigated using genetic methodologies approached in at least two different ways. Firstly, the history of a species that is entombed in the genome can be investigated (e.g. Lippe et al. 2006). However, the ability to reconstruct a species' past decreases when numbers decline and genetic variation is reduced. Another tactic is to sequence DNA from ancient and historical samples of a species, such as subfossil bones and museum specimens, to more directly examine the past. Knowledge of a species' past can be influential in its conservation, for example by elucidating its genetic variation and range before the impact of anthropogenic effects (e.g. Ross et al. 2006). Secondly, genetic markers can be used to target conservation issues in a contemporary timeframe. Applied problems can be addressed to aid current conservation management and future planning (e.g. Lada et al. 2007). This thesis is intellectually more centred on the second approach than the first, because the focal species is difficult to study by observational methods and it is in this area that genetics can be most useful to conservation. Nevertheless, the history of the species was also investigated to enable a thorough understanding of the remnant population in relation to its past.

Genetics as a Conservation Tool

Conservation is now essential to maintain the survival of the Earth's biodiversity. The impact of humans has increased the risk of extinction for many species, conservation aims to halt the extinction process. Molecular genetics provides useful tools to assist conservation initiatives and has been successfully used in many diverse ways (Allendorf and Luikart 2007). For example, genetic markers can aid decisions about what is to be conserved by defining species limits, management units and by identifying hybridisation (DeSalle and Amato 2004; Manel et al. 2005). Resources for conservation are often limited; phylogenetics can assist in the prioritisation of units to be conserved and in the allocation of funding (Redding and Mooers 2006). Molecular markers can help identify causes of population decline and monitor species under threat (Frankham et al. 2002; Schwartz et al. 2007). The effects of population decline on fitness can be investigated (Frankham 2005). Recovery of endangered populations can be aided by genetics, such as through 'genetic rescue', restoration and monitoring of the effects of translocations and captive management (Tallmon et al. 2004; McKay et al. 2005; Schwartz et al. 2007). Another application of genetics is wildlife forensics (Verma and Singh 2003).

To effectively conserve a species it is important to understand its biology, ecology, population dynamics and behaviour. Molecular techniques can be used to investigate genealogical relationships between individuals and populations. Understanding relatedness helps elucidate behaviour, mating systems, demography (such as effective population size), population structure and gene flow (Avise 2004). Landscape genetics is a new field that investigates how the genetic relatedness of individuals and populations is influenced by geographic and environmental features (Manel et al. 2003). Molecular sexing is very useful in conservation; disease detection and even diet can also be ascertained using genetics (Frankham et al. 2002). However, conservation genetics is most powerful when used in conjunction with ecology (DeYoung and Honeycutt 2005). Conservation geneticists and ecologists need to work closely together to maximise benefit for the species to be conserved (Wayne and Morin 2004).

Seabirds, the Quintessential Difficult to Study Group

Seabirds are top predators that influence ecological processes at sea and on land. They have been used as indicators to assess the condition of the oceanic environment (Moller et al. 2000). Marine birds are important to New Zealand, which has been described as the seabird 'capital' of the world. With 84 native marine bird species, of which 42% are endemic, New Zealand has more native seabird than landbird species (Taylor 2000).

Many seabirds are threatened, due mainly to the introduction of predators to breeding localities, interactions with fisheries and habitat destruction. Marine pollution and changes in oceanic temperature affecting prey also have a role. Seabirds are also at risk from oil spills, fires at nesting colonies and disease (Taylor 2000). Of 32 gadfly petrel *(Pterodroma)* species in the World Conservation Union (IUCN) redlist, 66% are threatened with extinction (IUCN 2006).

Knowledge of the biology and population dynamics of New Zealand seabirds is expanding but is still poorly understood (Wilson 2007). Seabirds are notoriously difficult to study. They are generally long-lived, have delayed maturity and are slow at reproducing (Schreiber and Burger 2002). Thus long-term studies are required to understand seabird ecology and biology, which require perseverance and can be expensive (Wooller et al. 1992). Petrels (Procellariiformes) are generally pelagic, spending most of their time foraging in the open ocean where they are difficult to observe (Brooke 2004). Gadfly petrels *(Pterodroma)* are often solitary at sea only coming to land to breed (usually colonially; Brooke 2004). Some gadfly petrels are nocturnal and inhabit underground burrows making observation difficult (Brooke 2004). They are generally sexually monomorphic and even separate species can look alike – especially when observed at sea or in flight (Marchant and Higgins 1990).

Genetic research can help overcome some of the difficulties encountered when studying seabirds. Molecular sexing allows the investigation of sex ratios (e.g. Moore et al. 2001; Weimerskirch et al. 2005). Genetic determination of parentage and relatedness can aid our understanding of behaviour and mating systems (e.g. Burg and Croxall 2006; Jouventin et al. 2007). Ancient DNA techniques can be used to investigate past population dynamics (Ritchie et al. 2004). Estimation of levels of genetic variation can examine demographic history and potential population bottlenecks (e.g. Moum and Arnason 2001; Genovart et al. 2007; Milot et al. 2007). Genetics can even be used to locate undiscovered individuals from a population or species (as is described in chapter four of this thesis). Hybridisation has also been studied using molecular techniques (e.g. Genovart et al. 2007). Phylogenetics can aid taxonomic classification (Kennedy and Page 2002; Penhallurick and Wink 2004) and phylogeographic

studies can identify population differentiation and philopatry in seabirds (for a review see Friesen et al. 2007).

Chatham Island Tāiko

The name 'Tāiko' is used to refer to the Chatham Island Tāiko (Tchāik, *Pterodroma magentae*). Other New Zealand birds (such as *Procellaria parkinsoni* and *Pterodroma inexpectata*) also have the name 'Tāiko' but are not mentioned in this dissertation. The Chatham Island Tāiko is a gadfly petrel and member of the family of tube-nosed seabirds 'Procellariidae'. It is classified as 'medium sized' (for gadfly petrels) with a length of ~40cm, wingspan of ~102cm and weight of 420-560g (Marchant and Higgins 1990). The Tāiko is pelagic, only coming to land for short periods during the breeding season (Imber, Taylor et al. 1994). When on land, the Tāiko is nocturnal and breeds in burrows. Only one egg is laid per year (without replacement; Taylor 2000). Tāiko burrows are thought to be restricted to the southwest of the main Chatham Island (Rēkohu / Wharekauri, where the Tāiko is endemic), mostly within the Tuku Nature Reserve (Aikman et al. 2001; Fig. 1.1). The life history traits of the Tāiko, its remote location and rarity, make this species difficult to observe and study.

The Tāiko is the world's most endangered seabird, classified as 'critically endangered' by the World Conservation Union (IUCN 2006). In New Zealand the Tāiko is one of the ten most endangered birds; ranked 'nationally critical' by the Department of Conservation, DOC (Hitchmough et al. 2007). Presently there are only 15 Tāiko pairs known to have bred in recent years. In addition, there are a number of non-breeding individuals (Imber et al. 2005) and the entire species is thought to comprise approximately 120 to 150 birds (Scofield 2004).

Tāiko are thought to have been very numerous in the past, but introduced predators and habitat destruction severely reduced the population size (Aikman et al. 2001). Moriori (the indigenous people of Chatham Islands / Rēkohu) introduced Kiore (Pacific rat, *Rattus exulans*) around 1100-1500 AD (King 2000; Fig. 1.1). Tāiko were however, still numerous when Europeans arrived in the early nineteenth century (Crockett 1994; Fig. 1.1). Europeans (Pākehā) introduced animals that are likely, if not known, predators of Tāiko eggs, chicks and adults. These include cats *(Felis catus)*, pigs *(Sus scrofa)*, dogs *(Canis familiaris)*, Norway / brown rats *(Rattus norvegicus)*, ship / bush / black rats *(Rattus rattus)* and later, Weka *(Gallirallus australi*, 1905) and Australian brushtail possums *(Trichosurus vulpecular*, 1911; Holmes 1993; Taylor 2000). By the late 1930s, large areas of land in the southwest of Chatham Island were cleared for farming destroying Tāiko habitat (Begg 1977; Fig. 1.1).

Sheep and cattle became feral, these animals are thought to trample petrel burrows and further destroy petrel habitat (Warham 1996). Predation is currently the major threat to Tāiko however other potential risks that could cause loss of Tāiko include flooding of burrows, disease and fishing interactions (Taylor 2000).

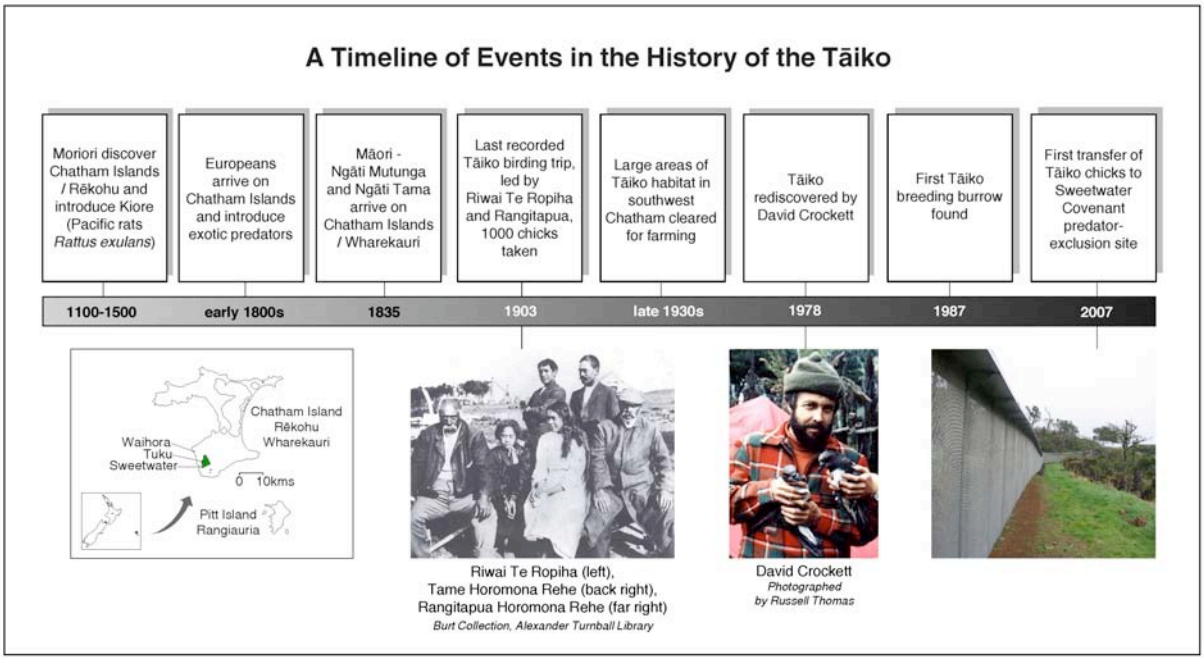

Figure 1.1 Timeline

Cultural Importance of the Tāiko (Tchāik to Moriori)

Moriori are the Tchakat henu, Tangata whenua tūturu ake, indigenous people of Chatham Island / Rēkohu (Waitangi Tribunal (WAI) 2001). They discovered Chatham Island (and neighbouring islands including Pitt / Rangiauria) around 1100-1500 AD (King 2000; Fig. 1.1). Moriori have conservation as part of their cultural tradition (Preece 1996). With no land mammals native to the Chatham Islands, the main diet of Moriori consisted of birds, fish and seals. Areas where wildlife and plants were collected were protected. For example, bird burrows were carefully preserved (Preece 1996). Moriori spirituality guided conservation practice through tapu (prohibition) and respect for tetua (gods, Richards 1972). The tetua Maru ensured people showed respect and care in the gathering and treatment of food (Preece 1996). When harvesting seabirds, the first bird killed was thrown over a cliff as a sacrifice to Maru (Holmes 1993). Moriori did not 'own' wildlife, but through respect of the tetua

gained rights to utilise it (WAI 2001). Birds were very important to Moriori as evidenced by the many rākau momori / rākau hokairo (tree carvings) depicting birds (Jefferson 1955). Birds were considered as kin (Skinner and Bauke 1928).

'Tāiko' is pronounced 'Tchāik' in the Moriori language (Skinner and Bauke 1928). The Tāiko was an important food source for Moriori (Sutton 1980). Large numbers of Tāiko bones have been uncovered at the Waihora village archaeological site (Sutton 1979; Fig. 1.1). The Tāiko young were extracted from burrows in March and April after the parents had finished feeding them (Skinner and Bauke 1928). Birding for Tāiko was highly ritualised. Sexual activity was prohibited the night before an expedition (Skinner and Bauke 1928). Karakii (prayers) were recited to the etchu (patron spirit) of the Tāiko the night before and during the journey (Skinner and Bauke 1928). Before birds were eaten taumaha (thanksgiving) was offered and the birds could apparently only be eaten inside the whare (house, Shand 1894). Seabirds including Tāiko were also preserved for long-term storage (Skinner and Bauke 1928).

The Tāiko was important not just as food for consumption but for cultural reasons. Manaakitanga is a central value that ensures visitors are well looked after (especially with regards to food), to maintain the mana (honour) of the host (Mead 2003). Moriori preserved whale meat underground, which was dug up to be part of feasts for maurahiri (visitors, Skinner and Bauke 1928). In the past, it is likely that preserved Tāiko were also used for this purpose. Seabird meat and fat is a delicacy called huahua (Shand 1911). Moriori held seabirds as mana kai. This refers to foods for which imi (tribes) were famous, which enhanced their mana (WAI 2001). Furthermore, seabirds were an important part of the feasts that were an essential part of tāhū (marriage ceremonies, Shand 1911). Some trade and exchange of resources took place (Sutton 1980), this may have included preserved Tāiko (Crockett 1994). Trips to bird for Tāiko were a family affair (King 2000) and no doubt served to strengthen kinship bonds. A famous Moriori man Tame Horomona Rehe (Tommy Solomon, Fig. 1.1, who has many descendants) was born on a Tāiko birding trip (King 2000). These concepts reinforce the importance of food items (including Tāiko) to the Moriori, for cultural reasons not just consumption.

Māori - Ngāti Mutunga on Chatham Island / Wharekauri are Tangata whenua iho (those who came after, WAI 2001). They arrived in 1835 with Ngāti Tama after hearing about the great abundance of fish and birds on the Chatham Islands from a Māori whaler named Pakiwhara (Hīroa 1949; Fig. 1.1). Māori took tohuk (Moriori priests) on birding trips, to placate the atua (tetua / gods) on their behalf, for both their safety and the survival of the species (WAI 2001). Māori also observed Moriori ritual for hunting albatross (King 2000) and therefore

probably Tāiko also. Many preserved 'mutton-birds' (petrels) and albatross were sent from the Chatham Islands to relatives in New Zealand (Aotearoa) to support those in the New Zealand wars (Aikman and Miskelly 2004). Chatham Island muttonbirds therefore were an important part of Māori heritage (WAI 2001) and probably included the Tāiko. The Tāiko was one of the species birded by Māori who inhabited Otonga in the southwest of Chatham Island (Begg 1977; Crockett 1994).

It is likely that Moriori muttonbirding was sustainable, since there were still large numbers when Europeans arrived in the Chatham Islands (Crockett 1994). Sustainable harvesting of other petrels has and continues to occur (Boersma and Parrish 1998; Brooke 2004). However, the decline in Tāiko numbers caused by introduced predators and destruction of habitat resulted in the cessation of harvesting. One of the last known birding trips for Tāiko was in 1903, led by Rangitapua Horomona Rehe and Riwai Te Ropiha, in which 1000 chicks were harvested (King 2000; Fig. 1.1).

The Tāiko is still a taonga (treasured) species to present day Chatham Islanders. Many are descendants and relatives of Tāiko birders such as Tame Horomona Rehe, Riwai Te Ropiha and Charles Seymour. The importance of the Tāiko in contemporary Chatham Island culture is evidenced by its location on Chatham Island dollar notes and the 2004 New Zealand commemorative five-dollar coin. It also features in artwork and souvenirs. The pioneering conservation efforts for birds such as the Tāiko and Black Robin have raised the international profile of the Chatham Islands (Seymour 2004). Landowners have donated reserves and formed covenants for conservation. For example, Manuel and Evelyn Tuanui donated the 1238ha Tuku Nature Reserve in 1984. Bruce and Liz Tuanui covenanted a further 200ha of adjacent forest and the 2ha Sweetwater Covenant, for Tāiko conservation (Aikman and Miskelly 2004; Fig. 1.1). Other landowners also provide access to their private land and support for Tāiko conservation. Chatham Islanders are members of the Tāiko Trust and Tāiko recovery group and play an active role in Tāiko conservation. The involvement of children and young people is encouraged (Emeny and Goomes 2007).

Tāiko History in Western Science

The identification and classification of petrel species on the Chatham Islands by early Western ornithologists and paleontologists has a chequered history. Confusion began when Archey and Lindsay (1924) documented a petrel described to them by Harry G. Blyth of Tuku Station, Chatham Island. They referred to this bird as the Chatham Island Petrel *OEstrelata*

axillaris (Forbes 1893), but it is likely they were instead providing the first scientific record of the Chatham Island Tāiko. Fleming (1939) investigated further. He had heard of a bird called 'Tāiko' that bred near the Tuku-a-Tamatea river. It was very rare but still thought to be surviving as Blyth had seen it several times in the previous two decades. Fleming recognised *Pterodroma axillaris* as a different species, because it had a different Māori name (Ranguru, note the genus name changed). Furthermore, he discovered numerous petrel skulls in sand dunes that were a few millimetres longer than *P. inexpectata* from mainland New Zealand. He knew *P. inexpectata* was also referred to as 'Tāiko' on the mainland so "tentatively" suggested the Tāiko was *P. inexpectata* (Fleming 1939). It wasn't until 1944 that he realised the skulls were in fact from a different and as yet unidentified species (Fleming 1944, 1953). The Tāiko remained without a Latin name until 1964, when Bourne suggested it was the same species as the Magenta Petrel, *Pterodroma magentae* (Bourne 1964).

The Magenta Petrel is a mysterious bird that was collected by one group of seamen during a single voyage. In 1867, while sailing the South Pacific Ocean aboard the H. I. M. S. '*Magenta'*, Italian naturalist Giglioli obtained the type specimen of an unusual seabird and named it the "Magenta Petrel". He later saw a few more of the same species near the Juan Fernandez Islands (Giglioli and Salvadori 1869). No other specimens of this bird were ever collected or even seen by the scientific community and so it was presumed extinct. Comparisons of the Magenta Petrel type specimen to bones and descriptions of the Chatham Island Tāiko led Bourne (1964) to suggest the birds were of the same species, *Pterodroma magentae.*

Rediscovery and Conservation of the Tāiko

The Chatham Island Tāiko was considered (by Western science) to have gone extinct in the early 1900's until it was amazingly rediscovered in 1978 (Crockett 1979, 1994). As a schoolboy, David Crockett had worked on the osteological collection of Canterbury Museum with the then vertebrate curator Ron Scarlett. Crockett surmised that many of the unidentified petrel bones in the Canterbury collection from the Chatham Islands could be that of the Tāiko. In 1952, Crockett wrote to Harry Blyth – a farmer on the Chatham Islands - who replied with information regarding a possible remaining colony of Tāiko at the Tuku. In 1970 Crockett went to the Chatham Islands to investigate and then every year thereafter. In 1973 he saw four Tāiko in flight. Few people believed him until 1978 when he (and the 'Tāiko Team') finally

captured two Tāiko (Crockett 1994; shown in Fig. 1.1). Crockett (now aged 71) established the Tāiko Trust and still plays a very active role in Tāiko conservation.

Once the Tāiko was rediscovered, conservation and research efforts were undertaken. However, it took 17 years of searching before the first active Tāiko burrow was discovered in 1987 (Fig. 1.1). The burrow was found by utilising radio telemetry methods (Imber, Crockett et al. 1994). Once burrows were found they were monitored and protected from predators. The Tāiko breeding season (September to May) was first reported in 1991 (Taylor 1991). This was an important milestone as it enabled better co-ordination of the predator control efforts and provided information on when was best to search for burrows. Tāiko burrows have continued to be discovered using radio telemetry, trained dogs and searches by personnel. Predator control was improved from 1996 onwards (Imber et al. 2005) and video monitoring of burrows has occurred since 1999 (Johnston et al. 2003).

It is thought that not all Tāiko breeding burrows have been discovered (Aikman et al. 2001). It is important that the locations of burrows are known so that Tāiko can be protected from introduced predators. Tāiko burrows are difficult to find because the native bush of southwest Chatham Island is very dense and burrows are usually isolated (Grant 1994). Expeditions occur approximately biannually to catch and track Tāiko. Tāiko are caught in flight at night using spotlighting techniques (Crockett 1994; Imber et al. 2005). A radio transmitter is attached and the bird is tracked using telemetry. Personnel (sometimes aided by trained dogs) then search the location identified by radio signals (Imber, Crockett et al. 1994; Imber et al. 2005). This is an expensive and time-consuming process.

A current conservation initiative of the Tāiko Trust is the establishment of a new Tāiko colony within a predator exclusion fence. In 2007 the entire cohort of Tāiko chicks was transferred to artificial burrows within the predator-free area (known as the Sweetwater Covenant, Fig. 1.1). This will continue for the next three years, after which fewer chicks will be translocated (DOC 2007). The chicks are transferred before they emerge from the burrow, in the expectation that they will be imprinted on the new site and return there to breed. A solar-powered sound system that broadcasts Tāiko calls has also been installed in order to lure adult Tāiko into the fenced area. The success of this exciting scheme will not be known until the juveniles return from sea aged four to six years old and breed at six to nine years (Imber et al. 2005).

Tāiko Research

Current understanding of Tāiko is growing but limited and there are a number of important conservation management issues regarding its biology and population dynamics that remain unanswered (Aikman et al. 2001). Since Tāiko are long-lived, perhaps 30-40 years, not even one cohort of Tāiko has been studied throughout its entire lifespan (Aikman et al. 2001). Some research on Tāiko has been opportunistic. For example, in 1996 a Tāiko corpse was found providing the first complete Tāiko specimen and allowing physiological examination. Intestinal structure was then used as one of many characters for taxonomy (Imber et al. 1998). Feather lice hosted by Tāiko have been identified and suggested a past close association with the Chatham Petrel *(Pterodroma axillaris*; Palma and Imber 2000).

More recent research on Tāiko has included the investigation of breeding and fledging behaviour of Tāiko using video monitoring of burrows (Johnston et al. 2003). A study of non-breeding behaviour was reported in 2005 (Imber et al.). Unpublished data has been collected by Department of Conservation staff and others on familial relationships (such as identifying parents and by inference, siblings) and behavioural activities of Tāiko (Aikman et al. 2001; G. A. Taylor and M. J. Imber unpubl. data). However, there are still gaps in knowledge of first-order relationships (both parents are not always identified) and more distant relationships are still unknown.

The Tāiko is an interesting species to research from a genetics perspective. A previous study using minisatellite DNA profiling has shown Tāiko to have surprisingly high levels of genetic variation, even higher than non-threatened New Zealand birds (Lambert and Millar 1995; Millar and Lambert 1999). This is unusual for endangered species (Frankham 1995; Spielman et al. 2004; for exceptions see Avise 2004). Usually, critically endangered species' level of genetic variation is low, limiting the ability of genetic markers to resolve differences between individuals. The Chatham Island Black Robin *(Petroica traversi)* is a good example (Ardern and Lambert 1997).

Thesis Rationale and Overview

Genetic research of the Chatham Island Tāiko can help understand relationships between individuals and groups, which can add to what is known about Tāiko biology, ecology and population processes. This information can then assist conservation management practices that aim to restore Tāiko to a self-sustaining population (Aikman et al. 2001).

This thesis is comprised of six research chapters. Chapter two examines the level of genetic variation in the Tāiko population. This chapter has been accepted for publication in Conservation Genetics. Lawrence HA, Taylor GA, Millar CD, Lambert DM (2008) High mitochondrial and nuclear genetic diversity in one of the world's most endangered seabirds, the Chatham Island Tāiko *(Pterodroma magentae).* Conservation Genetics *in press.*

The best method to examine changes in genetic variation over time (potentially due to reduction in population size) is by investigating the past population (Wisely et al. 2002). Chapter three describes ancient DNA research on Tāiko bones for that purpose and to investigate past geographic distribution. This chapter was submitted as a research manuscript to Heredity. Lawrence HA, Scofield RP, Crockett DE, Millar CD, Lambert DM (2008) Ancient genetic variation of the world's most endangered seabird. Heredity *under review.*

The potential for the existence of undiscovered Tāiko individuals and groups is evaluated in chapter four. This has important relevance to conservation. A manuscript based on chapter four has been accepted for publication in Conservation Biology. Lawrence HA, Taylor GA, Crockett DE, Millar CD, Lambert DM (2008) A new genetic approach to detecting individuals of rare and endangered species. Conservation Biology *accepted.*

Also of relevance to conservation initiatives is the identification of Tāiko sex and sex ratios, investigated in chapter five. This manuscript has been accepted for publication in the Journal of Avian Biology. Lawrence HA, Millar CD, Taylor GA, MacDonald LD, Lambert DM (2008) Excess of unpaired males in one of the world's most endangered seabirds, the Chatham Island Taiko *(Pterodroma magenate)*. Journal of Avian Biology *in press.*

On a more detailed level, knowledge of the relationships of Tāiko individuals can elucidate behaviour and understanding of the mating system. This was investigated using microsatellite DNA in chapter six, which will be submitted to Molecular Ecology. Lawrence HA, Taylor GA, Millar CD, Lambert DM (2008) Microsatellite DNA analysis reveals behaviour and familial relationships in the world's most endangered seabird. Molecular Ecology *in preparation.*

Chapter seven examines the relationship between the enigmatic Magenta Petrel and the Tāiko. It has been submitted to the Journal of Avian Biology. Lawrence HA, Millar CD, Imber MJ, Crockett DE, Robins JH, Scofield RP, Taylor GA, Lambert DM (2008) Solving the Mystery of the Magenta Petrel. Journal of Avian Biology *under review.*

To conclude, chapter eight is a discussion of the research findings, their potential use for conservation and possible future research. This thesis contains four appendices. Appendix A includes supplementary material specifically for chapter two. It includes details of the methods that are also relevant for chapter four and seven. Appendix B includes supplementary

material for chapter three only and appendix C contains methodological information relevant to chapter three and seven. Appendix D includes the genetic data.

References

Aikman H, Davis A, Miskelly C, O'Conner S, Taylor GA (2001) Chatham Island Tāiko Recovery Plan 2001-2011. Threatened Species Recovery Plan 36. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/TSRP36.pdf

Aikman H, Miskelly C (2004) Birds of the Chatham Islands. Department of Conservation, Wellington

Allendorf FW, Luikart G (2007) Conservation and the Genetics of Populations. Blackwell, Maden

Archey G, Lindsay C (1924) Notes on the birds of the Chatham Islands. Records of the Canterbury Museum 2:187-201

Ardern SL, Lambert DM (1997) Is the Black Robin in genetic peril? Molecular Ecology 6:21-28

Avise JC (2004) Molecular Markers, Natural History, and Evolution, 2 edn. Sinauer Associates, Massachusetts

Begg A (1977) Development of the Otonga land block, southwest coast, Chatham Islands. In: Working Papers in Anthropology, Archaeology, Linguistics, Māori Studies. Anthropology Department, University of Auckland, Auckland

Boersma PD, Parrish JK (1998) Threats to seabirds: research, education, and societal approaches to conservation. In: Avian Conservation: Research and Management (eds. Marzluff JM, Sallabanks R), pp. 237-260. Island Press, Washington DC

Bourne WRP (1964) The relationship between the Magenta Petrel and the Chatham Island Tāiko. Notornis 11:139-144

Brooke M (2004) Albatrosses and Petrels Across the World. Oxford University Press, Oxford

Burg TM, Croxall JP (2006) Extrapair paternities in Black-browed *Thalassarche melanophris*, Grey-headed *T. chrysostoma* and Wandering Albatross *Diomedea exulans* at South Georgia. Journal of Avian Biology 37:331-338

Crockett DE (1979) Rediscovery of the Chatham Island Tāiko solved a century-old mystery. Forest and Bird 13:8-13

Crockett DE (1994) Rediscovery of Chatham Island Tāiko *Pterodroma magentae*. Notornis (Supplement) 41:49-60

DeSalle R, Amato G (2004) The expansion of conservation genetics. Nature Reviews Genetics 5:702-712

DeYoung RW, Honeycutt RL (2005) The molecular toolbox: genetic techniques in wildlife ecology and management. Journal of Wildlife Management 69:1362-1384

DOC (Department of Conservation) (2007) A world first: Chatham Island Tāiko fledge from predator-proof site. In: The Chatham Islander 12 June 2007

Emeny G, Goomes T (2007) The moving of the Chatham Island Petrel and the endangered Tāiko. In: The Chatham Islander 13 March 2007

Fleming CA (1939) Birds of the Chatham Islands Part I. Emu 38:380-413

Fleming CA (1944) A petrel on the North Auckland mainland. New Zealand Bird Notes 1:58

Fleming CA (1953) Checklist of New Zealand Birds, 1 edn. Ornithological Society of New Zealand Inc., Wellington

Forbes HO (1893) A list of the birds inhabiting the Chatham Islands. Ibis 1893:521-546

Frankham R (1995) Conservation genetics. Annual review of genetics 29:305-327

Frankham R (2005) Genetics and extinction. Biological Conservation 126:131-140

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Conservation Genetics. Cambridge University Press, Cambridge

Friesen VL, Burg TM, McCoy KD (2007) Mechanisms of population differentiation in seabirds. Molecular Ecology 16:1765-1785

Genovart M, Oro D, Juste J, Bertorelle G (2007) What genetics tell us about the conservation of the critically endangered Balearic Shearwater. Biological Conservation 137:283-293

Giglioli HH, Salvadori T (1869) On some new Procellariidae collected during a voyage round the world in 1865-68. Ibis 5:61-68

Grant A (1994) Chatham Island Tāiko Recovery Plan 1994-2000. Department of Conservation, Wellington

HĪroa TR (1949) The Coming of the Māori. Whitcombe and Tombs, Wellington

Hitchmough R, Bull L, Cromarty P (comps) (2007) New Zealand Threat Classification System Lists 2005. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/sap236.pdf

Holmes D (1993) My Seventy Years on the Chatham Islands. Shoal Bay Press Ltd., Christchurch

Imber MJ, Crockett DE, Gordon AH, Best HA, Douglas ME, Cotter RN (1994) Finding the burrows of Chatham Island Tāiko *Pterodroma magentae* by radio telemetry. Notornis (Supplement) 41:69-96

Imber MJ, Taylor GA, Grant AD, Munn A (1994) Chatham Island Tāiko *Pterodroma magentae* management and research, 1987-1993: predator control, productivity, and breeding biology. Notornis (Supplement) 41:61-68

Imber MJ, Taylor GA, Tennyson AJD, Aikman HA, Scofield RP, Ballantyne J, Crockett DE (2005) Non-breeding behaviour of Magenta Petrels *Pterodroma magentae* at Chatham Island, New Zealand. Ibis 147:758-763

Imber MJ, Tennyson AJD, Taylor GA, Johnston P (1998) A second intact specimen of the Chatham Island Tāiko *(Pterodroma magentae)*. Notornis 45:247-254

IUCN (World Conservation Union) (2006) IUCN Red List of Threatened Species 2006. IUCN, Gland, Switzerland. Available from http://www.iucnredlist.org (accessed June 2007)

Jefferson C (1955) The dendroglyphs of the Chatham Islands. Journal of the Polynesian Society 64:367-441

Johnston RB, Bettany SM, Ogle RM, Aikman HA, Taylor GA, Imber MJ (2003) Breeding and fledging behaviour of the Chatham Tāiko (Magenta Petrel) *Pterodroma magentae* and predator activity at burrows. Marine Ornithology 31:193-197

Jouventin P, Charmantier A, Dubois M-P, Jarne P, Bried J (2007) Extra-pair paternity in the strongly monogamous Wandering Albatross *Diomedea exulans* has no apparent benefits for females. Ibis 149:67-78

Kennedy M, Page RDM (2002) Seabird supertrees: combining partial estimates of Procellariiformes phylogeny. Auk 119:88-108

King M (2000) Moriori A People Rediscovered, 2 edn. Penguin Books (NZ) Ltd., Auckland

Lada H, MacNally R, Taylor AC (2007) Genetic reconstruction of the population dynamics of a carnivorous marsupial *(Antechinus flavipes)* in response to floods. Molecular Ecology 16:2934-2947

Lambert DM, Millar CD (1995) DNA science and conservation. Pacific Conservation Biology 2:21-38

Lippe C, Dumont P, Bernatchez L (2006) High genetic diversity and no inbreeding in the endangered Copper redhorse, *Moxostoma hubbsi* (Catostomidae, Pisces): the positive sides of a long generation time. Molecular Ecology 15:1769-1780

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends in Ecology and Evolution 18:189-197

Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions with appropriate techniques. Trends in Ecology and Evolution 20:136-142

Marchant S, Higgins PJ (1990) Handbook of Australian, New Zealand and Antarctic birds. Oxford University Press, Melbourne

McKay JK, Christian CE, Harrison S, Rice KJ (2005) "How local is local?" - a review of practical and conceptual issues in the genetics of restoration. Restoration Ecology 13:432-440

Mead HM (2003) Tikanga Māori: Living By Māori Values. Huia, Wellington

Millar CD, Lambert DM (1999) Investigation of Sex and Genetic Relatedness in the Critically Endangered Chatham Island Tāiko Using DNA Technology. Institute of Natural Resources, Massey University, Palmerston North

Milot E, Weimerskirch H, Duchesne P, Bernatchez L (2007) Surviving with low genetic diversity: the case of albatrosses. Proceedings of the Royal Society B 274:779-787

Moller H, Frampton C, Hocken AG, McLean IG, Saffer V, Sheridan L (2000) The importance of seabird research for New Zealand. New Zealand Journal of Zoology 27:255-260

Moore PJ, Burg TM, Taylor GA, Millar CD (2001) Provenance and sex ratio of Black-browed Albatross, *Thalassarche melanophyrus*, breeding on Campbell Island, New Zealand. Emu 101:329-334

Moum T, Arnason E (2001) Genetic diversity and population history of two related seabird species based on mitochondrial DNA control region sequences. Molecular Ecology 10:2463-2478

Palma RL, Imber MJ (2000) Coexistence of two species of *Halipeurus* (Phthiraptera) on Chatham Island Tāiko *(Pterodroma magentae)* (Aves). New Zealand Journal of Zoology 27:229-232

Penhallurick J, Wink M (2004) Analysis of the taxonomy and nomenclature of the Procellariiformes based on complete nucleotide sequences of the mitochondrial cytochrome *b* gene. Emu 104:124-147

Preece AB (1996) Foreword. In: The Chatham Islands Heritage and Conservation, pp. 8-10. Canterbury University Press, Christchurch

Redding DW, Mooers AO (2006) Incorporating evolutionary measures into conservation prioritization. Conservation Biology 20:1670-1678

Richards R (1972) A tentative population distribution map of the Morioris [*sic*] of Chatham Island, circa 1790. Journal of the Polynesian Society 81:350-374

Ritchie PA, Millar CD, Gibb G, Baroni C, Lambert DM (2004) Ancient DNA enables timing of the Pleistocene origin and Holocene expansion of two Adelie Penguin lineages in Antarctica. Molecular Biology and Evolution 21:240-248

Ross JD, Arndt AD, Smith RFC, Johnson JA, Bouzat JL (2006) Re-examination of the historical range of the Greater Prairie Chicken using provenance data and DNA analysis of museum collections. Conservation Genetics 7:735-750

Schreiber EA, Burger J (eds) (2002) Biology of Marine Birds. CRC Press, Boca Raton

Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22:25-33

Scofield RP (2004) Population assessment of the endangered Chatham Island Tāiko. In: Third International Albatross and Petrel Conference, Montevideo, Uruguay

Seymour P (2004) Foreward. In: Birds of the Chatham Islands (eds. Aikman H, Miskelly C). Department of Conservation, Wellington

Shand A (1894) The Moriori people of the Chatham Islands, their traditions and history. Journal of the Polynesian Society 3:76-92

Shand A (1911) The Moriori people of the Chatham Islands, their history and traditions. The Polynesian Society of New Zealand, Wellington

Skinner HDB, Baucke W (1928) The Morioris. Bernice P. Bishop Museum, Honolulu

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Science USA 101:15261-15264

Sutton DG (1979) Island and coastal fowling strategies of the prehistoric Moriori. In: Birds of a Feather Osteological and Archaeological Papers from the South Pacific in Honour of R. J. Scarlett (ed. Anderson A), pp. 123-139. British Archaeological Reports, Oxford

Sutton DG (1980) A culture history of the Chatham Islands. Journal of the Polynesian Society 89:67-93

Tallmon DA, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. Trends in Ecology and Evolution 19:489-496

Taylor GA (1991) Report on the Chatham Island Tāiko and Chatham Island Petrel Recovery Programmes (1990/91). Threatened Species Occasional Publication 2. Department of Conservation NZ, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/TSOP02.pdf

Taylor GA (2000) Action Plan for Seabird Conservation in New Zealand Part A: Threatened Seabirds. Threatened Species Occasional Publication 16. Department of Conservation, Wellington http://www.doc.govt.nz/upload/documents/science-and-technical/TSOP16.pdf

Verma SK, Singh L (2003) Novel universal primers establish identity of an enormous number of animal species for forensic application. Molecular Ecology Notes 3:28-31

WAI (Waitangi Tribunal) (2001) Rēkohu: A Report on Moriori and Ngāti Mutunga Claims in the Chatham Islands. Waitangi Tribunal, Wellington

Warham J (1996) Petrels and man. In: The Behaviour, Population Biology and Physiology of the Petrels. Academic Press, London

Wayne RK, Morin PA (2004) Conservation genetics in the new molecular age. Frontiers in Ecology and the Environment 2:89-97

Weimerskirch H, Lallemand J, Martin J (2005) Population sex ratio variation in a monogamous long-lived bird, the Wandering Albatross. Journal of Animal Ecology 74:285-291

Wilson K-J (2007) The State of New Zealand's Birds 2006: Special Report Seabirds. Ornithological Society of New Zealand Inc., Wellington

Wisely SM, Buskirk SW, Fleming MA, McDonald DB, Ostrander EA (2002) Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. Journal of Heredity 93:231-237

Wooller RD, Bradley JS, Croxall JP (1992) Long-term population studies of seabirds. Trends in Ecology and Evolution 7:111-114

Chapter Two

High Mitochondrial and Nuclear Genetic Diversity in the World's Most Endangered Seabird

Abstract

Interpreting the levels of genetic diversity in organisms with diverse life and population histories can be difficult. The processes and mechanisms regulating this diversity are complex and still poorly understood. However, endangered species typically have low genetic variation as a consequence of the effects of genetic drift in small populations. In this study genetic variation was examined in the Chatham Island Tāiko (Tchāik, *Pterodroma magentae*), the world's most endangered seabird. The Tāiko has a very small population size of between 120-150 individuals, including just 8-15 breeding pairs. Surprisingly high mitochondrial and nuclear genetic diversity was found in this critically endangered long-lived species. It is likely that the present Tāiko population has retained a significant proportion of its past genetic diversity. However, it is also possible that undiscovered birds are breeding in unknown areas, which could increase the population size estimate. Importantly, from a conservation perspective, it is shown that the high level of variation is unlikely to be maintained in the future since chicks currently being born have only a limited number of the mitochondrial DNA haplotypes found in adults. Reduced genetic variation will mean that our ability to infer past events and the population history of Tāiko using genetics could soon be lost and the power to

determine, for example, parentage and other close order relationships, will be diminished. Therefore the maintenance of genetic diversity in future generations is an important consideration for conservation management of the Tāiko.

Introduction

Interpreting the levels of genetic diversity in organisms with diverse life and population histories can be difficult. This is because the mechanisms and processes that regulate this diversity are complex and still poorly understood. Therefore, determining the level of genetic variation in an endangered long-lived seabird and comparing this to other avian species is helpful especially in the context of known population history, demography and life history characteristics. From a conservation perspective, priority should be given to the quantification and characterisation of levels of genetic diversity for all endangered species. This assessment of diversity can assist us in evaluating the likely genetic effects of future population changes and can be a guide in conservation management that aims to maintain current levels of diversity (Roques and Negro 2005).

Endangered species typically have low genetic variation, especially when compared with closely related taxa that are not threatened (Ardern and Lambert 1997; Spielman et al. 2004; for exceptions see Avise 2004). Reductions in genetic variation associated with population bottlenecks effectively cause genetic homogenisation that therefore limits our ability to recover historical information. Moreover, a reduction in genetic diversity will also reduce the utility of genetic markers in resolving contemporary conservation issues such as identifying parentage and more distant relationships. These can in turn be used to elucidate an endangered species' behaviour, mating system, philopatry, spatial genetic structure and population history.

The world's most endangered seabird is the Chatham Island Tāiko (Tchāik, *Pterodroma magentae*, IUCN 2006). This burrowing gadfly petrel is endemic to the main Chatham Island (Rēkohu/Wharekauri) located 860km east of New Zealand (Aikman et al. 2001). The Tāiko was considered extinct by Western science until 1978 and is now ranked 'critically endangered' (Crockett 1994; IUCN 2006). Presently there are only 15 Tāiko pairs known to have bred in recent years. In addition, there are a number of non-breeding individuals and the entire species is thought to comprise only 120-150 birds (Scofield 2004). Many of the life history characteristics of Tāiko that are known are shared with other related seabird species. These include delayed breeding (seven years of age being the youngest recorded, G. A.

Taylor pers. comm.), low reproductive rate (only one egg is laid per year), social monogamy, long-term partnerships and a long lifespan (perhaps 30-40 years, Aikman et al. 2001). However the Tāiko is difficult to observe and study because it is rare, pelagic and only comes to land during the breeding season where it is nocturnal and inhabits underground burrows in a single remote location (Aikman et al. 2001). Therefore, the relationship between individuals is very difficult to determine by observation alone. A variety of molecular methods can provide an alternative approach to this problem but require the presence of a degree of genetic variation in the population. Knowledge of relatedness is important in understanding mating systems and behaviour such as philopatry.

Fledgling survival is critical in order to prevent the extinction of the Tāiko. Tāiko chicks are vulnerable to predation, so intense predator control is maintained around burrows and fledgling flight paths during the breeding season (Aikman et al. 2001). A predator exclusion fence has been built around the site of an extinct Tāiko colony and management plans for Tāiko include the translocation of chicks to artificial burrows within this safer area (Aikman et al. 2001). Such a transfer would occur before chicks emerge from burrows so that they will potentially become imprinted on the new site (as with other petrels, Gummer 2003). It is anticipated that the birds' philopatric behaviour will result in Tāiko returning to the predator-excluded area after they come back from the sea to breed. In addition, Tāiko will be attracted to the new colony from flight by broadcasting of vocalisations (Aikman et al. 2001).

Mitochondrial DNA (mtDNA) is a useful marker to evaluate genetic diversity in Tāiko because it has been used extensively in studies of birds, thereby enabling comparison to other avian species. Mitochondrial DNA is haploid and generally maternally inherited so is more susceptible to genetic drift and bottlenecks (Avise 2004). Therefore, although useful, mtDNA variation can underestimate overall genomic variation and is not necessarily representative of nuclear diversity (Zhang and Hewitt 2003). Furthermore, Bazin et al. (2006) have also recently questioned the link between mtDNA diversity, population size and history in a study in which they compared a very diverse array of animal taxa. However, in contrast, when more closely related species are compared a positive correlation between population size, history and mtDNA diversity was found (e.g. Hughes and Hughes 2007) indicating the continued utility of the marker.

The different population history, life history and reproductive ecology of animals can affect genetic diversity and rates of decline (Kuo and Janzen 2004). To contribute to our understanding of this, genetic diversity was evaluated in a sample comprising almost the entire known Tāiko population. To assess genetic diversity in the Tāiko the mitochondrial cytochrome *b* gene and both copies of a fragment of the duplicated control region domain I

were sequenced and multilocus minisatellite DNA techniques were used. These genetic markers could be used to aid management initiatives important for the conservation of the critically endangered Tāiko.

Methods

Blood samples were collected from almost every Tāiko caught since 1996 ($N = 148$, $N = 117$ were used in this study). DNA was extracted by proteinase K digestion and a modified version of the phenol/chloroform method (Sambrook et al. 1989). The complete mitochondrial cytochrome *b* gene was amplified using the polymerase chain reaction (PCR) with primers L14863 (Nunn et al. 1996) and HTāikoThr2 5'-GGTTTTACAAGACCAATGTT-3' (designed by Leon Huynen; PCR conditions, product purification and sequencing details in appendix A). Sequencing primers included L14863, HTāikoThr2 and internal primers LCytB432 5'-TGAGGACAAATATCATTCTGAGG-3' and HCytB571 5'-GGAAGGTGAGGTGGATTAAGG-3'.

Duplication of the mitochondrial control region is known in Procellariiform species (Abbott et al. 2005). Sequencing of domain I of the control region in Tāiko revealed double peaks in sequencing electropherograms at some nucleotide sites, suggesting duplication also exists in Tāiko. The possible existence of an amplified nuclear pseudogene was tested for and excluded (details in appendix A). Both copies of a 315bp region in domain I were amplified and sequenced (PCR conditions, product purification and sequencing details in appendix A). These regions were designated fragments 1 and 2. Fragment 1 was amplified by PCR with primers F1AF 5'- AATGGCCCATGTGCGGTTGT-3' and HCRPtb 5'-CTAGGGGTGTAGGGGGAAAG-3', fragment 2 with F2AF 5'-AATGGTCTATGTGTGGGTGC-3' and HCRPtb. Sequences were deposited in the National Institutes of Health (U.S.A) genetic sequence database GenBank®. Accession numbers are EU302268 to EU302460. Sequences were aligned using Sequencher™ version 4.2.2 (GeneCodes). Selective neutrality of mtDNA haplotypes was tested for using Tajima's *D* calculated in Arlequin 3.1 (Tajima 1989; Excoffier et al. 2005).

A multilocus DNA profiling pilot study was performed to determine which restriction enzyme / probe combination to use to achieve the optimal resolution of fragments (C. D. Millar unpubl. data). Subsequent minisatellite DNA profiling was performed as in Millar et al. (1994), with *Alu I* digestions in combination with the probe $(CA)_n$ (Ellegren 1991; C. D. Millar unpubl. data).

Results

Cytochrome *b* and domain I control region haplotypes were determined for 90 Tāiko adults and 66 chicks born since intense predator control and monitoring began in 1993. Ten individuals fall into both categories since they were caught as chicks and returned as adults. In total, 117 individuals were sequenced and haplotypes for others were inferred from known maternal relationships.

A total of 12 polymorphic sites defined 10 unique haplotypes in Tāiko for cytochrome *b* (Table 2.1). Fragment 1 contained 32 polymorphic sites defining 20 unique haplotypes. Fragment 2 contained 21 polymorphic sites defining 19 haplotypes. When the cytochrome *b*, fragment 1 and fragment 2 sequences are combined, 21 haplotypes are apparent (Table 2.1). Only 11 of these haplotypes were recorded among the 66 chicks (Table 2.1).

Mitochondrial DNA diversity was compared between Tāiko, seabird and other avian species, with respect to phylogeny and conservation status (Table 2.2). The number of cytochrome *b* and control region domain I (CR I) haplotypes were divided by the number of individuals included in the study and number of variable nucleotides per 100bp were calculated to enable ease of comparison with mitochondrial DNA diversity in Tāiko. For DNA samples collected prior to 1998, levels of minisatellite DNA bandsharing were calculated between pairwise combinations of Tāiko adults (mean 0.17 ± 0.032 SE, range 0 - 0.25, N = 19; C. D. Millar unpubl. data).

Table 2.1 Variable nucleotide sites of 21 mitochondrial DNA haplotypes in Tāiko *(Pterodroma magentae)*.
Dots indicate identity to the reference sequence.

Haplotype	Number of Tāiko Adults	Number of Tāiko Chicks*	Cytochrome *b* (1143bp) Nucleotide Position	Fragment 1 Control Region Domain I (315bp) Nucleotide position	Fragment 2 Control Region Domain I (315bp) Nucleotide position
			1 2 4 5 7 7 7 7 8 8 9 1	1 1 1 1 1 1 1 1 1 1 1 2 2 2 2 2 2 2 2 2	1 1 1 1 1 1 2 2 2 2 2 2 2 2
			4 0 3 7 1 2 3 4 1 4 1 2	1 1 5 6 6 6 7 7 7 9 9 9 9 0 0 1 1 3 4 5 6 6 6 9 0 0 2 2 3 3 5 7	5 5 7 7 7 8 9 0 3 4 5 6 9 0 0 2 2 3 3 5 7
			5 4 2 1 6 0 8 1 9 0 5 2	1 4 8 3 5 7 5 7 9 4 5 6 9 1 6 0 5 9 6 5 1 2 4 4 6 7 3 9 0 1 0 9	3 6 3 7 8 9 4 6 9 6 6 4 4 6 7 3 9 0 1 0 9
AZZ	3	0	C T G G C A C C A C T G	T T C A T C A T C C C T T G G G G C A C T C T C T C C G C C A C	C C A C T G C G C A A C C T C C G C C A C
AYY	18*	10	. . . . .	. . T . . . . . A . R . . . . . T . A . T . T	. T . T . A . . . . . T . A . T . T
AXX	1	0	. . . . .	. . T . . . . . A . . . . T . A . T . T	. . . T . . . . . T . . T T A . T . T
AWW	6	0	. . . . .	. . T . . . . C . . . . . A . . . . T . A . T G T	. . . T C A . . . . . T . A . T G T
AVQ	10	18	. . . . .	. . T . . . . . T . . C A A . . . . . T . . . . . . A . . T	. . . T . . . . . T . . . . . A . . T
AUU	2	0	. . . . .	. . T . . . . C . . . . . A . . . . T . A . T . T	. . . T . . . . . T . A . T . T
ATQ	1	1	. . . . .	. . . . . T . . C . A . A . . . T . . . . . . A . . T	. . . T . . . . . T . . . . . A . . T
ATT	1*	1	. . . . .	. . . . . T . . C . A . A . . . T . . . . . . A . . T	. . . T . A . . . . . A . . T
ASS	4	5	. . . . .	. . T . . . . C . . . . . A . A . . . . . . T . . . T G .	. . . T . A . . . . . T . . . T G .
ARR	2	0	. . . . .	. . . . . C A . . . . . T . A . . T	. . . T . . . . . T . A . . T
BBB	8	7	. . A	. . . . . A . . . . . T . . . . A . . T	. . . T . . . . . T . . . . A . . T
CCC	3	4	T . . . . . T . G T C .	C C T . C . . C . . . . . A A A A . . . C T . T . . . . A . G .	. . . T . . . . . T T . . . . A . G .
DDD	4	0	T . . . . . T	. . T . . . . . T . . A A . . T G . . . . . A . . .	. . . T . . T . T G . . . . . A . . .
EEE	6	2	T . . . T	. . T . . . . . T . C A A . . . G . . . . . A . . .	. . . T . . T . . G . . . . . A . . .
FFF	3	2	. . . . . T	. . T G . . C . . . . . A . . . . . T . . . . A . . T	T . . T . A . . . . G T T . . . . A . . T
GKM	11	9	T . . . . . A	. . T . . T . . . . . A A . . . G . . . . . T . A . . .	. . . T . . T . . G . . . . . T . A . . .
GLM	2	7	T . . . . . A	. . T . . . . . T . . . A . . . . G . . . . . T . A . . .	. . . T . . T . . G . . . . . T . A . . .
HHH	1	0	T	. . T . . . . . T . . . A . A . . G . . . . . A . . .	. . G T . . T . . G . . . . . A . . .
III	1	0	T C	. . T . . . . . T . . A A A . . G . C T C . . . . . A . . .	. . . T . . T . . . . . A . . .
JOO	2	0	. . . A	. . T . . . . . T . . A . . . . . C T . . . T G .	. . . T . . . A . . . . . C T . . . T G .
JPP	1	0	. . . A	. . T . . . . . A A . . . . . C T . . . T G .	. . . T . . . . . C T . . . T G .
TOTAL	21	90	66		

* Two of the adult haplotypes were inferred from their chick's haplotype, many chick haplotypes were inferred from their mother's haplotype
Note: Ten individuals fall into both adult and chick categories since they were first caught as chicks and have returned to the colony as adults

Table 2.2 Comparisons of Tāiko *(Pterodroma magentae)* mitochondrial DNA diversity with Procellariiform, Charadriiform and other avian species of varying conservation status (continued on next page)

Phylogenetic Groupings	Common Name	Scientific Name	Conservation Status*	Sample Size *(N)*	Marker Sequenced **	Number of Sampled Haplotypes / *N*	Number of Variable Sites per 100bp	Reference
Procellarii -formes	**Tāiko**	***Pterodroma magentae***	**Critically Endangered**	**90**	Cyt-*b* (1143bp)	**0.11**	**1.05**	
	Herald Petrel	*Pterodroma heraldica*	Least Concern	33	Cyt-*b* (307bp)	0.15	2.28	Brooke and Rowe 1996
	European Storm Petrel	*Hydrobates pelagicus*	Least Concern	65	Cyt-*b* (910bp)	0.12	1.32	Cagnon et al. 2004
Chara -drii -formes	Marbled Murrelet	*Brachyramphus marmoratus*	Endangered	47	Cyt-*b* (1045bp)	0.30	-	Friesen et al. 1996
	Least Tern	*Sterna antillarum*	Least Concern	51	Cyt-*b* (362bp)	0.06	0.55	Whittier et al. 2006
	Ancient Murrelet	*Synthliboramphus antiquus*	Least Concern	58	Cyt-*b* (306bp)	0.09	1.31	Pearce et al. 2002
Other Avian Orders	Black-faced Spoonbill	*Platalea minor*	Endangered	87	Cyt-*b* (548bp)	0.01	0	Yeung et al. 2006
	Great Bustard	*Otis tarda*	Vulnerable	66	Cyt-*b* (292bp)	0.03	0.34	Pitra et al. 2000
	Ferruginous Pygmy-Owl	*Glaucidium brasilianum*	Least Concern	103	Cyt-*b* (5' 899bp)	0.29	3.34	Proudfoot et al. 2006
	Loggerhead Shrike	*Lanius ludovicianus*	Least Concern	72	Cyt-*b* (200bp)	0.04	1.00	Mundy et al. 1997

* as recognised by the World Conservation Union (IUCN 2006)

** Cyt-*b*: cytochrome *b*, CR I: control region domain I, CR II: control region domain II

Table 2.2 continued

Phylogenetic Groupings	Common Name	Scientific Name	Conservation Status*	Sample Size *(N)*	Marker Sequenced **	Number of Sampled Haplotypes / *N*	Number of Variable Sites per 100bp	Reference
Procellariiformes	**Tāiko**	***Pterodroma magentae***	**Critically Endangered**	**90**	CR I (315bp) **F1** CR I (315bp) **F2**	**0.22** **0.21**	**10.16** **6.67**	
	Black-browed Albatross	*Thalassarche melanophrys*	Endangered	56	CR I (219bp)	0.75	24.66	Burg and Croxall 2001
	Grey-headed Albatross	*Thalassarche chrysostoma*	Vulnerable	50	CR I (220bp)	0.78	18.18	Burg and Croxall 2001
	Wandering Albatross	*Diomedea exulans*	Vulnerable	42	CR I (234bp)	0.76	8.12	Alderman et al. 2005
Charadrii -formes	Ancient Murrelet	*Synthliboramphus antiquus*	Least Concern	58	CR I (494bp)	0.22	3.04	Pearce et al. 2002
	Razorbill	*Alca torda*	Least Concern	123	CR I (300bp)	0.35	12.67	Moum and Arnason 2001
	Common Murre	*Uria aalge*	Least Concern	79	CR I (266bp)	0.37	10.53	Moum and Arnason 2001
	Sooty Tern	*Sterna fuscata*	Least Concern	55	CR I (315bp)	0.85	18.10	Avise et al. 2000
Other Avian Orders	White-headed Duck	*Oxyura leucocephala*	Endangered	46	CR I & II (574bp)	0.07	0.35	Munoz-Fuentes et al. 2005
	Houbara Bustard	*Chlamydotis undulata*	Vulnerable	73	CR (854bp)	0.45	5.04	Idaghdour et al. 2004
	Spanish Imperial Eagle	*Aquila adalberti*	Vulnerable	60	CR I (315bp)	0.05	0.63	Martinez-Cruz et al. 2004
	Imperial Eagle	*Aquila heliaca*	Vulnerable	34	CR I (315bp)	0.18	1.90	Martinez-Cruz et al. 2004
	Blue Chaffinch	*Fringilla teydea*	Near Threatened	66	CR I (288bp)	0.05?	2.78?	Pestano et al. 2000
	Plain Pigeon	*Columba inornata*	Near Threatened	42	CR I (315bp)	0.12	1.59	Young and Allard 1997
	Red Kite	*Milvus milvus*	Near Threatened	105	CR I (315bp)	0.10	3.17	Roques and Negro 2005
	Common Chaffinch	*Fringilla coelebs*	Least Concern	42	CR I (300bp)	0.40	7.33	Marshall and Baker 1997
	Loggerhead Shrike	*Lanius ludovicianus*	Least Concern	93	CR I (200bp)	0.04	1.50	Mundy et al. 1997
	California Gnatcatcher	*Polioptila californica*	Least Concern	64	CR (315bp)	0.14	1.90	Zink et al. 2000
	Northern Goshawk	*Accipiter gentilis*	Least Concern	49	CR I (315bp)	0.08	0.95	Sonsthagen et al. 2004
	Sandhill Crane	*Grus canadensis*	Least Concern	73	CR I & II (650bp)	0.15	10.30	Rhymer et al. 2001
	Japanese Hodgson's Hawk-Eagle	*Spizaetus nipalensis oreientalis*	Species Least Concern	93	CR I (315bp)	0.24	5.40	Asai et al. 2006

* as recognised by the World Conservation Union (IUCN 2006)
** Cyt-*b*: cytochrome *b*, CR I: control region domain I, CR II: control region domain II

Discussion

Mitochondrial DNA diversity in Tāiko can be usefully compared with that in other seabirds and in other avian groups. These comparisons include species with different degrees of phylogenetic relatedness to Tāiko and varying conservation status. In the case of cytochrome *b*, Tāiko had only slightly less variation than that found in the two other Procellariiformes (tube-nosed seabirds), but more than some Charadriiform seabirds and most other avian species (Table 2.2). The conservation status of Charadriiform and other avian species having less variation than Tāiko included species that are endangered, vulnerable and in the least concern category (as ranked by the World Conservation Union, IUCN 2006).

The number of control region domain I (CR I) haplotypes in Tāiko was less than that recorded for most other seabirds analysed. However, the number of variable sites in CR I in Tāiko is more than that found in two other seabirds (Table 2.2). In addition, Tāiko had more CR I variation than most of the non-seabird avian species (Table 2.2). The species of Procellariiformes (albatross) compared with Tāiko were either endangered or vulnerable (IUCN 2006). However, these albatross species are comprised of more than one population and have many thousands of breeding pairs (e.g. Burg and Croxall 2001) compared with the Tāiko which has 8-15. The Tāiko did have an equivalent number of CR I haplotypes and many more variable sites than one non-threatened seabird (the Ancient Murrelet, Table 2.2). Moreover, Tāiko had more CR I diversity than many threatened or near threatened avian species and most of the avian species of 'least concern' used for comparison (Table 2.2). Therefore, mitochondrial genetic variation in Tāiko is relatively high especially considering it's extremely low numbers. Furthermore, the extensive sampling of Tāiko provided a rare opportunity to obtain a very robust measure of mitochondrial DNA diversity in a vertebrate species.

The values of bandsharing between the multilocus profiles of Tāiko individuals are generally very low in comparison to similar studies of New Zealand birds (Lambert and Millar 1995 Table 2) and low in comparison to other petrels (e.g. Short-tailed Shearwaters *Puffinus tenuirostris* 0.298 ± 0.017 SE, Austin et al. 1993; Leach's Storm-Petrels *Oceanodroma leucorhoa* 0.58 ± 0.059 SD, Mauk et al. 1995). Hence, the low levels of bandsharing between Tāiko indicate that this endangered species has a large amount of nuclear genetic diversity. Therefore, findings indicate that both mitochondrial and nuclear genetic variation are reasonably high in the Tāiko compared with other avian species, including non-threatened birds. This is surprising since endangered species typically have low genetic variation (e.g. Ardern and Lambert 1997; Spielman et al. 2004; for exceptions see Avise 2004).

When assessing genetic variation, it is important to examine both mitochondrial and nuclear diversity for the same species. In the case of Tāiko, mitochondrial DNA markers are useful because they enable a direct comparison with many other avian species. Evaluating diversity using nuclear genetic markers is also important so that both genomes are represented (Zhang and Hewitt 2003). The level of genetic variation in Tāiko was higher in multilocus DNA profiles than in the mitochondrial DNA (as measured by comparison with other avian species).

Selection may occasionally favour the retention of high genetic diversity in small and isolated populations (Kaeuffer et al. 2007). No evidence was found for selection on mtDNA haplotypes (Tajima's D, $P > 0.05$ for each region). Another potential explanation for the reasonably high level of genetic variation in the critically endangered Tāiko is the real possibility that undiscovered birds are breeding elsewhere, this could increase the population size estimate. The mitochondrial diversity may be useful to detect the existence of any undiscovered birds. For example, if unique haplotypes are detected in birds caught in flight and not found in those caught on the ground this would suggest there are more Tāiko breeding in undiscovered areas.

Another hypothesis for the high genetic diversity observed in Tāiko could be that the current Tāiko population retains a significant proportion of past genetic diversity. Relatively high genetic variation in a rare species can indicate that decline in numbers is recent (Moritz 1994). Population contraction over a large number of generations may be required for loss of genetic variation, but this effect is influenced by a species' life history traits (Lippe et al. 2006). Long generation times and delayed sexual maturity can slow the loss of genetic variation (Kuo and Janzen 2004). Tāiko are long-lived, reaching approximately 30-40 years, become sexually mature at a minimum of seven years and have overlapping generations (Aikman et al. 2001; G. A. Taylor pers. comm.). Furthermore, past demography could also be a contributing factor in the loss of diversity (Goossens et al. 2005). The Tāiko was previously very numerous, once the most abundant burrowing seabird on Chatham Island (Aikman et al. 2001).

The speed of demographic fluctuation can also have an effect on genetic variation, e.g. more genetic diversity may be retained when a decline in population numbers is gradual (Lippe et al. 2006). It seems likely that the decline in Tāiko numbers was initially gradual and probably the result of the arrival of the first humans on Chatham Island. However, the population almost certainly underwent a steep decline in the early parts of the last century as predation and habitat clearance intensified. Knowledge of the history of the Tāiko population allows inferences to be made from these genetic data. Moriori (the indigenous people)

discovered the Chatham Islands/Rēkohu around 1100-1500 AD (King 2000). These people harvested Tāiko/Tchāik for food and introduced Kiore (Pacific rat, *Rattus exulans)*, which may have negatively impacted Tāiko numbers (Crockett 1994). Europeans arrived in the early nineteenth century and introduced many animals that are known predators of Tāiko (Crockett 1994). Also, by the late 1930s, large areas of Tāiko habitat were cleared for farming (Begg 1977). Records indicate that Tāiko were still reasonably abundant at the beginning of the 20th century (Crockett 1994). For example, in 1903 1000 Tāiko chicks were harvested (King 2000). Since only a proportion of the population breed, there still would have been a significant population of Tāiko at this time, around a century ago.

It is therefore likely that the surprisingly high level of genetic variation in the living Tāiko population is at least in part due to the significant retention of past diversity. High genetic diversity despite substantial population decline has been observed in other long-lived species with delayed sexual maturity (e.g. the ornate box turtle *Terrapene ornate*, Kuo and Janzen 2004; orang-utan *Pongo pygmaeus*, Goossens et al. 2005; copper redhorse *Moxostoma hubbsi*, Lippe et al. 2006). This suggests that the life history traits of long-generation time and delayed maturity can buffer loss of genetic variation, especially when decline is recent. Hence our findings support the population genetic theory prediction that severe bottlenecks may not drastically reduce the genetic diversity when they last for a small number of generations (Amos and Balmford 2001).

However, low population growth rate can cause bottlenecks to persist, during which time genetic diversity will be further eroded (Allendorf and Luikart 2007). The Tāiko has a long life span and slow reproduction that reduces the rate of genetic drift but also slows recovery from a small population size. The effects of the population bottleneck have not yet been detected in the adult generation, but the current high level of genetic variation may be transient and is not likely to be observed in future generations. Intense predator control and monitoring of Tāiko began in 1993; as a result the number of Tāiko chicks fledged has substantially increased. However, the mtDNA diversity in this next generation is severely reduced. Of the 21 mitochondrial DNA haplotypes identified in the adult population, only half (11) are represented in the next generation (i.e. chicks born since 1993). Six of the adult haplotypes are only found in male birds, so will not be passed to the next cohort. Four adult haplotypes are found in females not known to breed. Therefore, from a scientific and management perspective it is important that genetic markers are used to study the Tāiko immediately while the population still retains sufficient genetic variation. More of the genetic history will be lost in the next generation and the power to determine parentage and close order relationships might be very significantly diminished just one generation from now.

A valuable conservation management tactic is to lessen average kinship in order to maintain genetic diversity (Avise 2004). This is relevant to a current conservation management initiative, i.e. the establishment of a new Tāiko colony within a predator-excluded site. Chicks will be translocated so they will potentially become imprinted on the new site and return there to breed once mature. Chicks can be chosen in order to maximise the retention of genetic variation described in this study and reduce founder effects in the future colony.

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References

Abbott C, Double MC, Trueman JWH, Robinson A, Cockburn A (2005) An unusual source of apparent mitochondrial heteroplasmy: duplicate mitochondrial control regions in *Thalassarche* albatrosses. Molecular Ecology 14:3605-3613

Aikman H, Davis A, Miskelly C, O'Conner S, Taylor GA (2001) Chatham Island Tāiko Recovery Plan 2001-2011. Threatened Species Recovery Plan 36. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/TSRP36.pdf

Alderman R, Double MC, Valencia J, Gales RP (2005) Genetic affinities of newly sampled populations of Wandering and Black-browed Albatross. Emu 105:169-179

Allendorf FW, Luikart G (2007) Conservation and the Genetics of Populations. Blackwell, Maden

Amos W, Balmford A (2001) When does conservation genetics matter? Heredity 87:257-265

Ardern SL, Lambert DM (1997) Is the Black Robin in genetic peril? Molecular Ecology 6:21-28

Asai S, Yamamoto Y, Yamagishi S (2006) Genetic diversity and extent of gene flow in the endangered Japanese population of Hodgson's Hawk-Eagle, *Spizaetus nipalensis*. Bird Conservation International 16:113-129

Austin JJ, Carter RE, Parkin DT (1993) Genetic evidence for extra-pair fertilisations in socially monogamous Short-tailed Shearwaters, *Puffinus tenuirostris* (Procellariiformes: Procellariidae), using DNA fingerprinting. Australian Journal of Zoology 41:1-11

Avise JC, Nelson WS, Bowen BW, Walker D (2000) Phylogeography of colonially nesting seabirds, with special reference to global matrilineal patterns in the Sooty Tern *(Sterna fuscata)*. Molecular Ecology 9:1783-1792

Avise JC (2004) Molecular Markers, Natural History, and Evolution, 2nd edn. Sinauer Associates, Massachusetts

Bazin E, Glemin S, Galtier N (2006) Population size does not influence mitochondrial genetic diversity in animals. Science 312:570-572

Begg A (1977) Development of the Otonga land block, southwest coast, Chatham Islands. In: Working Papers in Anthropology, Archaeology, Linguistics, Māori Studies. Anthropology Department, University of Auckland, Auckland

Brooke MDL, Rowe G (1996) Behavioural and molecular evidence for specific status of light and dark morphs of the Herald Petrel *Pterodroma heraldica*. Ibis 138:420-432

Burg TM, Croxall JP (2001) Global relationships amongst Black-browed and Grey-headed Albatrosses: analysis of population structure using mitochondrial DNA and microsatellites. Molecular Ecology 10:2647-2660

Cagnon C, Lauga B, Hemery G, Mouches C (2004) Phylogeographic differentiation of Storm Petrels *(Hydrobates pelagicus)* based on cytochrome *b* mitochondrial DNA variation. Marine Biology 145:1257-1264

Crockett DE (1994) Rediscovery of Chatham Island Tāiko *Pterodroma magentae*. Notornis (Supplement) 41:49-60

Ellegren H (1991) Fingerprinting birds' DNA with a synthetic polynucleotide probe $(TG)_n$. Auk 108:956-981

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50

Friesen VL, Piatt JF, Baker AJ (1996) Evidence from cytochrome *b* sequences and allozymes for a 'new' species of Alcid: the Long-billed Murrelet *(Brachyramphus perdix)*. Condor 98:681-690

Goossens B, Chikhi L, Jalil MF, Ancrenaz M, Lackman-Ancrenaz I, Mohamed M, Andau P, Bruford MW (2005) Patterns of genetic diversity and migration in increasingly fragmented and declining orang-utan *(Pongo pygmaeus)* populations from Sabah, Malaysia. Molecular Ecology 14:441-456

Gummer H (2003) Chick translocation as a method of establishing new surface-nesting seabird colonies: a review. DOC Science Internal Series 150. Department of Conservation, Wellington http://www.doc.govt.nz/upload/documents/science-and-technical/dsis150.pdf

Hughes AL, Hughes MAK (2007) Coding sequence polymorphism in avian mitochondrial genomes reflects population histories. Molecular Ecology 16:1369-1376

Idaghdour Y, Broderick D, Korrida A, Chbel F (2004) Mitochondrial control region diversity of the Houbara Bustard *Chlamydotis undulata* complex and genetic structure along the Atlantic seaboard of North Africa. Molecular Ecology 13:43-54

IUCN (World Conservation Union) (2006) IUCN Red List of Threatened Species 2006. IUCN, Gland, Switzerland. Available from http://www.iucnredlist.org (accessed June 2007)

Kaeuffer R, Coltman DW, Chapuis J-L, Pontier D, Reale D (2007) Unexpected heterozygosity in an island Mouflon population founded by a single pair of individuals. Proceedings of the Royal Society B 274:527-533

King M (2000) Moriori A People Rediscovered, 2nd edn. Penguin Books (NZ) Ltd, Auckland

Kuo C-H, Janzen FJ (2004) Genetic effects of a persistent bottleneck on a natural population of ornate box turtles *(Terrapene ornata)*. Conservation Genetics 5:425-437

Lambert DM, Millar CD (1995) DNA science and conservation. Pacific Conservation Biology 2:21-38

Lippe C, Dumont P, Bernatchez L (2006) High genetic diversity and no inbreeding in the endangered copper redhorse, *Moxostoma hubbsi* (Catostomidae, Pisces): the positive sides of a long generation time. Molecular Ecology 15:1769-1780

Marshall HD, Baker AJ (1997) Structural conservation and variation in the mitochondrial control region of Fringilline finches *(Frinfilla* spp.) and the Greenfinch *(Carduelis chloris)*. Molecular Biology and Evolution 14:173-184

Martinez-Cruz B, Godoy JA, Negro J (2004) Population genetics after fragmentation: the case of the endangered Spanish Imperial Eagle *(Aquila adalberti)*. Molecular Ecology 13:2243-2255

Mauk RA, Waite TA, Parker PG (1995) Monogamy in Leach's Storm-Petrel: DNA-fingerprinting evidence. Auk 112:473-482

Millar CD, Anthony I, Lambert DM, Stapleton PM, Bergmann CC, Bellamy AR, Young EC (1994) Patterns of reproductive success determined by DNA fingerprinting in a communally breeding oceanic bird. Biological Journal of the Linnean Society 52: 31-48

Moritz C (1994) Applications of mitochondrial DNA analysis in conservation: a critical review. Molecular Ecology 3:401-411

Moum T, Arnason E (2001) Genetic diversity and population history of two related seabird species based on mitochondrial DNA control region sequences. Molecular Ecology 10:2463-2478

Mundy NI, Winchell CS, Woodruff DS (1997) Genetic differences between the endangered San Clemente Island Loggerhead Shrike *Lanius ludovvicianus mearnsi* and two neighbouring subspecies demonstrated by mtDNA control region and cytochrome *b* sequence variation. Molecular Ecology 6:29-37

Munoz-Fuentes V, Green AJ, Negro JJ, Sorenson MD (2005) Population structure and loss of genetic diversity in the endangered White-headed Duck, *Oxyura leucocephala*. Conservation Genetics 6:999-1015

Nunn GB, Cooper J, Jouventin P (1996) Evolutionary relationships among extant albatrosses (Procellariiformes: Diomedeidae) established from complete cytochrome-*B* gene sequences. Auk 113:784-801

Pearce RL, Wood JJ, Artukhin Y (2002) Mitochondrial DNA suggests high gene flow in Ancient Murrelets. Condor 104:84-91

Pestano J, Brown RP, Rodriguez F, Moreno A (2000) Mitochondrial DNA control region diversity in the endangered Blue Chaffinch, *Fringilla teydea*. Molecular Ecology 9:1421-1425

Pitra C, Lieckfeldt D, Alonso JC (2000) Population subdivision in Europe's Great Bustard inferred from mitochondrial and nuclear DNA sequence variation. Molecular Ecology 9:1165-1170

Proudfoot GA, Honeycutt RL, Slack RD (2006) Mitochondrial DNA variation and phylogeography of the Ferruginous Pygmy-Owl *(Glaucidium brasilianum)*. Conservation Genetics 7:1-12

Rhymer JM, Fain MG, Austin JE, Johnson DH, Krajewski C (2001) Mitochondrial phylogeography, subspecific taxonomy, and conservation genetics of Sandhill Cranes *(Grus canadensis*; Aves: Gruidae). Conservation Genetics 2:203-218

Roques S, Negro JJ (2005) MtDNA genetic diversity and population history of a dwindling raptorial bird, the Red Kite *(Milvus milvus)*. Biological Conservation 126:41-50

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York

Scofield RP (2004) Population assessment of the endangered Chatham Island Tāiko. In: Third International Albatross and Petrel Conference, Montevideo, Uruguay

Sonsthagen SA, Talbot SL, White CM (2004) Gene flow and genetic characterization of Northern Goshawks breeding in Utah. Condor 106:826-836

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Science USA 101:15261-15264

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585-595

Whittier JB, Leslie JR DM, Van Den Bussche RA (2006) Genetic variation among subspecies of Least Tern *(Sterna antillarum)*: implications for conservation. Waterbirds 29:176-184

Yeung CK-L, Yao C-T, Hsu Y-C (2006) Assessment of the historical population size of an endangered bird, the Black-faced Spoonbill *(Platalea minor)* by analysis of mitochondrial DNA diversity. Animal Conservation 9:1-10

Young DL, Allard MW (1997) Conservation genetics of the Plain Pigeon *Columba inornata* in Puerto Rico and the Dominican Republic. Molecular Ecology 6:877-879

Zhang D-X, Hewitt GM (2003) Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. Molecular Ecology 12:563-584

Zink RM, Barrowclough GF, Atwood JL, Blackwell-Rago RC (2000) Genetics, taxonomy, and conservation of the threatened California Gnatcatcher. Conservation Biology 14:1394-1405

Chapter Three

Ancient DNA Study of the Past Genetic Diversity and Breeding Distribution of the Tāiko

Abstract

Sequencing of DNA from ancient and historical samples (such as subfossil bones and museum specimens) can allow the past population history of a species to be investigated. This approach can provide a direct reference point for estimates of change in genetic variation over time. In addition, such ancient and historical samples can help assess the past breeding distribution of species. Furthermore, ancient in combination with modern genetic data can provide an invaluable perspective on a threatened species that is in decline. In this study, ancient DNA from a range of Chatham Island Tāiko (Tchāik, *Pterodroma magentae*) subfossil bones was sequenced for regions of the mitochondrial cytochrome *b* gene. Eight haplotypes were revealed: four were unique to the subfossil bones and four corresponded to those found in the modern Tāiko population. Surprisingly, despite the critically endangered status of the Tāiko, no reduction in genetic diversity was observed between ancient samples ($N = 44$) and modern adult Tāiko ($N = 90$), as measured by mitochondrial haplotype diversity and effective population size. The modern population has however, lost four haplotypes present in the ancient populations. Using mitochondrial DNA (mtDNA) analysis of subfossil bones sites of possible past Tāiko breeding colonies outside of the existing range of the species were

identified. It is also shown that the remnant breeding population in the southwest of Chatham Island has retained a large proportion of the genetic variation in extinct Tāiko populations, but that some of this diversity could be lost in just one generation from now.

Introduction

Any reduction in the genetic diversity of a species can be the result of a severe decline in population size (Spielman et al. 2004). Comparisons between the levels of genetic diversity in an endangered species to that of an ecologically similar species can be used to infer if such a decrease in variation has occurred (e.g. Wisely et al. 2002). However, this approach is potentially problematic because it assumes identical demographic history of the two species being compared (Matocq and Villablanca 2001). The best method of investigating changes in genetic variation over time is to use ancient DNA (aDNA) derived from samples of the population before it declined. This then provides a direct reference point (Wisely et al. 2002). Using such an approach, studies have compared diversity in pre-bottleneck samples to 'modern' samples (e.g. Chan et al. 2005; Leonard et al. 2005). One such study was even able to compare Pleistocene to Holocene diversity changes in an extinct species, the Beringian steppe bison (Shapiro et al. 2004). Some studies have discovered that the change in genetic diversity over a suspected bottleneck was less than expected (e.g. Paxinos et al. 2002; Miller and Waits 2003). Such results could only have been obtained by an examination of ancient DNA. In addition to past genetic diversity, the past distribution of species can also be investigated using DNA from ancient and/or historical samples (e.g. Ritchie et al. 2004; Ross et al. 2006). Identifying any contraction in a threatened species range is of importance for its conservation; ancient and historical DNA can be usefully employed for this purpose (Ross et al. 2006).

Genetic diversity in the modern population of the now critically endangered Chatham Island Tāiko (Tchāik, *Pterodroma magentae*) has been found to be relatively high in comparison to seabirds and other avian species (chapter two). It seems likely that the past genetic diversity has been retained in the extant population because the severe decline in Tāiko numbers occurred last century, only a few generations ago (chapter two). Direct sequencing of aDNA from samples of the species before its dramatic decline would elucidate this theory. The Tāiko is only ever known to have bred on the main island in the Chatham group (Aikman and Miskelly 2004; Fig. 3.1; details in appendix B). The species is thought to have been very numerous in the past, the most common of the burrowing petrels on Chatham

Island (Aikman and Miskelly 2004). However, the distribution of past Tāiko breeding populations on the main Chatham Island is unknown. This could be investigated if genetic structure is evident in aDNA from widely distributed Tāiko bone samples.

Tāiko bones have been discovered from many locations around the main Chatham Island, including the northern and eastern regions, primarily from sand dunes (Crockett 1994; Fig. 3.1). However, many inland areas are unsuitable for bone preservation, due to acidity, peat deposits (and fires therein) and widespread swamps (Millener 1996). Debate exists about whether dune bones are remains from breeding populations or from beach-wrecked birds (i.e. birds washed up on the shore, Worthy and Holdaway 2002), skua *(Catharacta* and *Stercorarius* species) refuse deposits (G. A. Taylor pers. comm.) or birds transported by Moriori (Millener 1999). Moriori (the indigenous people of Chatham Island/Rēkohu) harvested juvenile Tāiko from breeding burrows for food ("muttonbirded", Sutton 1979a). Birds were preserved and may have been transported around the island (Crockett 1994). Consequently, the presence of juvenile Tāiko bones in a location does not necessarily indicate a historical breeding site.

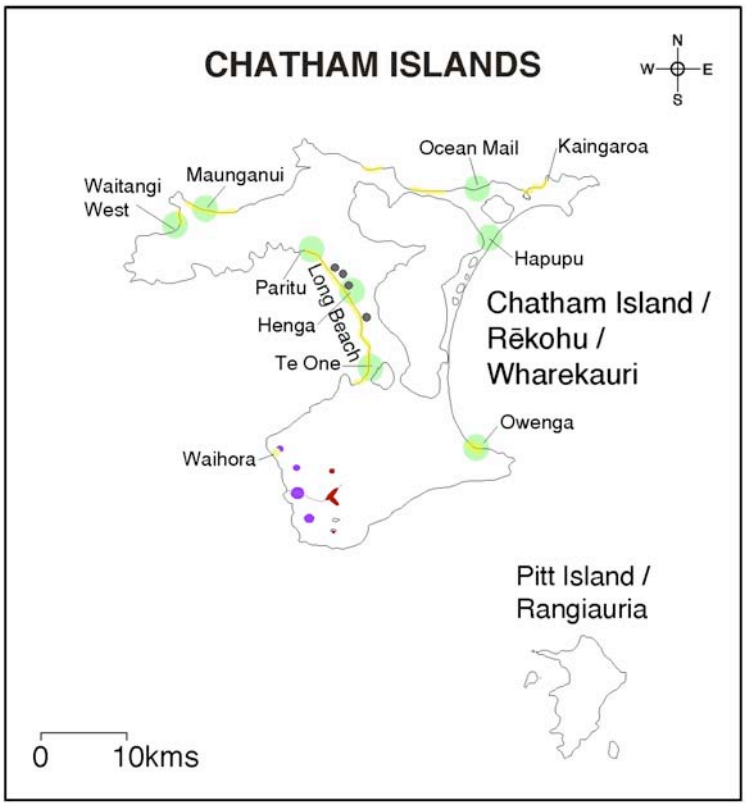

Figure 3.1 Possible Tāiko distribution. Sites where subfossil Tāiko bones found by others (in yellow, Crockett 1994) and in this study (green). Current Tāiko burrow locations (red) and extinct colonies are shown (purple, Sutton 1979a; D. E. Crockett pers. comm.). Possible extinct Tāiko colonies identified by Millener (1999) are indicated by grey circles.

Atkinson and Millener (1990) have suggested that Tāiko were once widely distributed across the main Chatham Island, but the southwest of Chatham Island is generally accepted as the main breeding area (Crockett 1994). Crockett identified the locations of Tāiko colonies in the southwest of Chatham Island that were very large in the past – of which only one remains today (D. E. Crockett pers. comm.; Fig. 3.1). Egg remains have been found outside of the southwest area (at Paritu; Fig. 3.1) that could be from Tāiko (pers. obs.; G. A. Taylor and R. P. Scofield pers. comm.), this may provide supporting evidence for other past breeding sites. Nestling bones found with eggshell fragments suggest past Tāiko breeding colonies at Long Beach (Millener 1999; Fig. 3.1). Tāiko bones (and in some cases complete skeletons) found at Long Beach have been radiocarbon dated to between ca.2050 and ca.3300 CAL BP (Millener 1999), long before humans reached Chatham Island (about 900 years ago, King 2000).

Tāiko were an important food source for Moriori living at the Waihora village in the 16th century (Sutton 1980; Fig. 3.1). Tāiko bones make up half of the species assemblages uncovered at some midden sites (human refuse deposits). These sites were likely to be highly specialised seasonal stations that were located near a Tāiko breeding colony, within 2km of the village (Sutton 1979a; Fig. 3.1). Inhabitants of Waihora were mostly self-sufficient, but there is evidence for some exchange with people from other areas for tools (Sutton 1982) and perhaps eels, lampreys and inanga (Sutton 1989). Therefore, it is possible that Tāiko were used in exchange and transported further north, but evidence is lacking.

Mitochondrial DNA from ancient Tāiko was sequenced to investigate levels of past genetic diversity in Tāiko and the past geographic distribution of breeding colonies. Ancient DNA was extracted from Tāiko bones collected from around Chatham Island and the archaeological sites around Waihora (Fig. 3.1). Mitochondrial DNA haplotypes were determined by sequencing of three regions of the cytochrome *b* gene totalling 311bp. These were compared to the haplotypes of the modern Tāiko population (chapter two). This comparison allows investigation of whether the modern Tāiko population has retained or lost its past genetic diversity. In addition, the distribution of haplotypes may allow determination of whether the Tāiko bred in areas other than the southwest of Chatham Island where the modern population is located.

Methods

Bone Samples

Tāiko bones were collected from the surface of sand dunes around main Chatham Island and obtained from Canterbury Museum (Fig. 3.1; details in appendix B, Tables B.1 and B.2). These bones could be up to 7000 years old (Millener 1999). Tāiko bones were also obtained from the Otago Museum (details in appendix B, Table B.3). These bones were collected from midden sites around Waihora and are approximately 260-450 years old (Sutton 1976; Sutton 1979b; Fig. 3.1).

aDNA Extraction Methods

DNA extractions were performed in a physically isolated dedicated ancient DNA laboratory. Appropriate measures were taken to prevent contamination (details in appendix C). The surface of the subfossil bone material was removed using sterile fine-grained sandpaper. A section was cut from the centre of the bone (with the aim to preserve morphology as much as possible) using a Dremel® hand-tool. Bone fragments of 15mg to 400mg were ground into a fine powder using a sterile mortar and pestle. The bone powder was decalcified and digested in 5ml of extraction buffer (10mM Tris-HCl pH8, 10mM NaCl, 500mM EDTA pH8), with 10μl of 10% sodium dodecyl sulfate (SDS), 30μl of 200mg/ml dithiothreitol (DTT) and 50μl of 50mg/ml proteinase K. Extraction negative controls were included at a ratio of 1:5 or 1:7 with aDNA extractions. Samples were incubated rotating overnight at 50°C.

Tubes were subsequently centrifuged at 2197 x g for 20mins in a MSE Mistral 2000 swing-bucket centrifuge. The supernatant was collected and samples extracted twice with phenol (for 30 minutes each), then once with chloroform: isoamyl alcohol (25:1) for 5 minutes. Samples were concentrated in Vivaspin 6 Ultrafiltration Spin Columns (VivaScience, U. K.) 5ml at a time at 2197 x g. Columns were washed twice with 5ml of ultraPURE™ distilled water (Gibco) then the 200μl supernatant was collected. Forty-two samples that did not initially amplify with PCR were subsequently purified using a QIAamp® DNA Mini Kit (Qiagen).

aDNA Amplification and Sequencing

Polymerase chain reaction (PCR) primers were designed to amplify regions of cytochrome *b* known to be variable in the modern Tāiko population: L14863 (Nunn et al. 1996) and HCytB21-55 5'-GGGGTGGGTAGGTCAATTAAG-3'; LCytB432-571 5'-TGAGGACAAATATCATTCTGAGG-3' and HCytB432-571 5'-GGAAGGTGAGGTGGATTAAGG-3'; and LCytB679-780 5'-CCCATTCCATCCCTACTTCA-3'

and HCytB 679-780 5'-TGGGGAGGTGTAATTAATGGA-3'. The reaction mixture for PCR contained 2-4μl of DNA extract, 1X PCR Buffer (Invitrogen), 1.5mM – 3mM $MgCl_2$ (Invitrogen), 200μM of each dNTP (Bioline), 2mg/ml bovine serum albumin (GibcoBRL), 0.2μM of each primer (Invitrogen), 1 – 2 units of Platinum® Taq DNA polymerase (Invitrogen) and ultraPURE™ distilled water to a final volume of 20μl. Negative and positive controls were included in each set of PCRs. Amplification was carried out in a BIO-RAD iCycler, with the following PCR profile: hot start 94°C for 2 min, then 10 cycles of 94°C for 20 seconds (sec), 55°C for 30 sec, 72°C for 30 sec; then 50 cycles of 94°C for 20 sec, 52°C for 30 sec, 72°C for 30 sec; then a final extension at 72°C for 10 mins. For amplification from some samples the annealing temperatures were reduced to 52°C and 50°C in the first and second set of cycles (respectively).

The PCR products were purified using a QIAquick® PCR Purification kit (Qiagen) then sequenced on an Applied Biosystems 3730 DNA analyser by the Allan Wilson Centre Genome Service. Sequences were compared to the reference sequence i.e. the most common haplotype found in the modern Tāiko population, haplotype A. If any base differentiated from the reference sequence at a site not known to be variable in the modern population, a minimum of two additional PCR amplifications were performed and sequenced. Sequencing artefacts were identified and excluded (details in appendix C). Sequences were aligned and edited using Sequencher™ version 4.2.2 (GeneCodes). Consensus sequences will be deposited in the GenBank® database. A haplotype network was constructed manually. Haplotype diversity was calculated according to Nei (1987) using DnaSP version 4.10.9 (Rozas et al. 2003). Female effective population size *(N_{ef})* was estimated by the equation: $\theta = 2N_{ef}\mu$ (Wright 1931; Fu 1994), from the ancient and modern samples (adults and chicks, data from chapter two). The population size parameter, theta θ, was estimated based on nucleotide diversity using the minimum number of mutations model of four possible nucleotides per site (Tajima 1996; Rozas et al. 2003). The mutation rate, μ, is a product of the substitution rate per site per year and the generation time. The rate of divergence used was 9×10^{-9} bp^{-1} $year^{-1}$ (i.e. 0.9% per million years using a Kimura two-parameter correction), calculated for cytochrome *b* in intermediate-sized Procellariidae (Nunn and Stanley 1998). Tāiko generation time was estimated as the average age of breeding females assuming that mortality is the same across different ages. The estimate was based on a lifespan of 30 years (Aikman et al. 2001) and age at first breeding of seven years (G. A. Taylor pers. comm.). The generation time estimate for Tāiko was therefore 19 years.

Replication

DNA from three Tāiko bones was extracted, amplified and purified for sequencing in another aDNA facility by Judith H. Robins (University of Auckland). Bones were ground as described above and DNA was extracted using the method described in Matisoo-Smith et al. (1997). The PCR protocol was as described above.

Results and Discussion

DNA was successfully extracted, amplified and sequenced for all three cytochrome *b* regions from a total of 44 out of 104 bones (42%). This rate is similar to that of other ancient DNA studies (Stiller et al. 2006 and references therein). These regions identify eight haplotypes in the modern Tāiko population (chapter two - note haplotypes G, H and I are indistinguishable using these three smaller regions). Four of these eight modern haplotypes were found in the ancient Tāiko bones, in addition to four new haplotypes (Table 3.1). Each new haplotype is unique, i.e. found in only one individual and is defined by a single substitution (Table 3.1, Fig. 3.2).

Table 3.1 Variable sites in cytochrome *b* defining haplotypes in modern and ancient Tāiko

Haplotype	**Cytochrome *b* (1143bp) Nucleotide Position**											**Population**
		4	5	5	6	7	7	7	7	7	7	
	4	3	4	7	8	0	1	2	2	3	4	
	5	2	1	1	7	4	6	0	4	8	1	
A	**C**	**G**	**G**	**G**	**T**	**T**	**C**	**A**	**G**	**C**	**C**	Both
B	.	A	.	.	.	.	.	.	.	.	.	Both
C	T	.	.	.	.	.	.	.	.	T	.	Modern Only
D	T	.	.	.	.	.	.	.	.	.	T	Modern Only
E	T	.	.	.	.	.	T	.	.	.	.	Modern Only
F	.	.	.	.	.	.	.	T	.	.	.	Both
G/H/I	T	.	.	.	.	.	.	.	.	.	.	Both
J	.	.	.	A	.	.	.	.	.	.	.	Modern Only
K	.	.	.	.	.	.	.	.	A	.	.	Ancient Only
L	.	.	.	.	.	C	.	T	.	.	.	Ancient Only
M	.	.	A	.	.	.	.	.	.	.	.	Ancient Only
N	.	.	.	.	C	.	.	.	.	.	.	Ancient Only

. indicate identity with reference sequence (haplotype A)
Nucleotide position is relative to the entire Cytochrome *b* sequence. The regions sequenced in this case are nucleotide positions 1 to 53, 429 to 580, 677 to 782 (totalling 311bp).

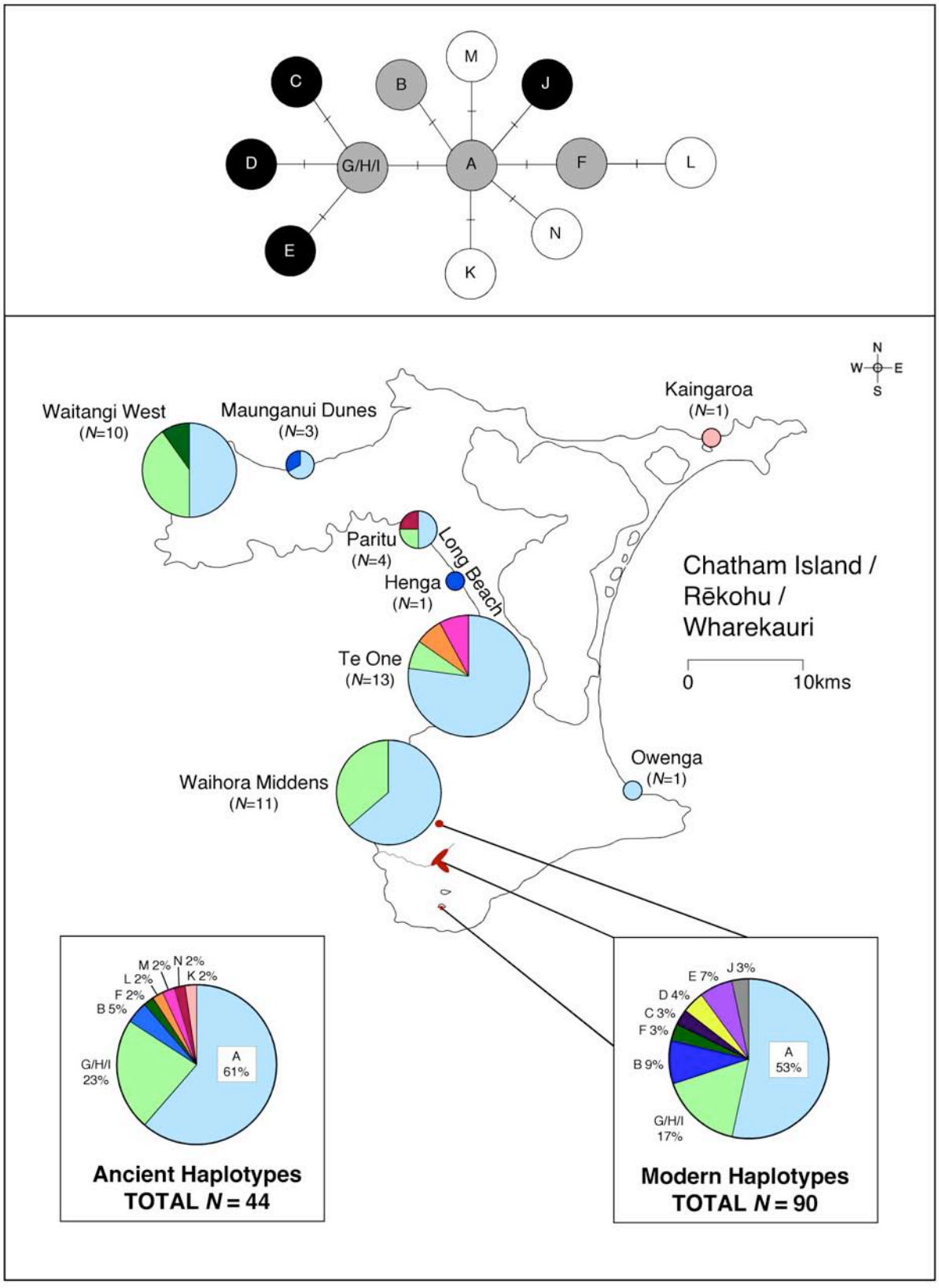

Figure 3.2 Network of cytochrome *b* mitochondrial DNA haplotypes of Tāiko that includes both modern and ancient samples (grey), modern samples only (black) and ancient samples only (white). A dash represents a single nucleotide base change. The geographic distribution of modern and ancient haplotypes is also shown.

The novel ancient haplotypes are found in bones from Kaingaroa (1), Paritu (1) and Te One (2, Fig. 3.2). The haplotype network is star-like, with almost all rare haplotypes radiating from common haplotypes (A and G/H/I) and differing from them by a single substitution (Fig. 3.2). This pattern can be indicative of a past population expansion (Slatkin and Hudson 1991). The haplotype diversity for the 44 aDNA sequences was 0.580 $\pm$ 0.072 SD. The equivalent haplotype diversity (i.e. for the 311bp) in modern adult samples was 0.678 $\pm$ 0.047 SD and 0.653 $\pm$ 0.048 SD for the modern chick samples. Therefore, the range of haplotype diversity overlaps and is not significantly different. The estimated values of θ were 0.00324 for modern samples of adults (N = 90), 0.00284 for modern samples of chicks (N = 66) and 0.00232 for ancient samples (N = 44). The effective female population size was estimated to be 9474 for modern adult Tāiko and 6784 for ancient Tāiko. Therefore N_{ef} is slightly higher for modern samples and indicates there has been no significant reduction in mtDNA cytochrome *b* variation. The effective female population size for chicks was 8304 showing a reduction of over 1000 in just one generation. However, these estimates of modern female effective population size are not related to current actual population size, which are orders of magnitude smaller. They are instead estimates of the number of breeding females when the population size was constant and not in decline.

The distribution of aDNA haplotypes indicates the geographic distribution of past Tāiko breeding populations. Novel haplotypes were found in Tāiko bones from the central west coast of Chatham Island. Two unique haplotypes were found at Te One, the closest dune site to where the current Tāiko colony and the colonies extirpated within the past few hundred years are located (Fig. 3.2). An additional unique haplotype was found at Paritu, at the northern end of Long Beach. Sites along Long Beach were proposed as possible past Tāiko breeding colonies by Millener (1999) on the basis of immature Tāiko bones being found in these areas and dated to pre-Moriori times (Fig. 3.1). The locations of the novel haplotypes also suggests past Tāiko breeding colonies along Long Beach (Fig. 3.2). A unique haplotype was discovered in a bone from Kaingaroa, the only bone representing this site (Fig. 3.2). The northeast region of Chatham Island may also have been the site of a past Tāiko breeding colony.

If a distinct breeding Tāiko colony was present in the northwest region of Chatham Island, novel haplotypes may be expected from the Waitangi West and Maunganui dune sites. However, this was not observed despite quite comprehensive sampling (i.e. 13 bones, equivalent to the number sampled from Te One). A Tāiko bone (not included in this study) found at Waitangi West was dated to a period after Moriori discovery of the island (Millener 1999). It is therefore possible that Tāiko bones found in the northwest region of Chatham

Island were transported by Moriori, or derive from beach-wrecked birds. If a Tāiko breeding colony was present in the northwest, results suggest there was gene flow with other colonies. Taken together, the results of this study suggest that the southwest of Chatham Island was the main Tāiko breeding area, or that there was gene flow between other past breeding Tāiko colonies.

Tāiko were very numerous in the past, so a higher genetic diversity than that of the very small modern population could be expected. However, with eight haplotypes each, both the modern population (N = 90 adults) and the ancient bones (N = 44) have the same haplotype diversity. This supports the theory that the modern Tāiko population has retained a large proportion of past diversity (chapter two). However, some variation has been lost since four haplotypes present in the ancient populations are not represented in the modern population. The ancient samples used in this study were a mixture of bones hundreds to thousands of years old, so the Tāiko population has retained a reasonably high level of genetic variation for a long period of time. However, the decline in the number of mitochondrial DNA haplotypes in the Tāiko chicks over just one generation (i.e. from eight in the adults to six in the chicks) suggests the Tāiko population will lose some of its mitochondrial variation in a very short period (chapter two). The level of genetic variation in Tāiko maintained over potentially thousands of years could now be lost in a matter of decades.

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References

Aikman H, Davis A, Miskelly C, O'Conner S, Taylor GA (2001) Chatham Island Tāiko Recovery Plan 2001-2011. Threatened Species Recovery Plan 36. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/TSRP36.pdf

Aikman H, Miskelly C (2004) Birds of the Chatham Islands. Department of Conservation, Wellington

Atkinson IAE, Millener PR (1990) An ornithological glimpse into New Zealand's pre-human past. In: Acta XX Congressus Internationalis Ornithologici (ed. Bell BD), pp. 127-192. New Zealand Ornithological Congress Trust Board, Christchurch

Chan YL, Lacey EA, Pearson OP, Hadly EA (2005) Ancient DNA reveals Holocene loss of genetic diversity in a South American rodent. Biology Letters 1:423-426

Crockett DE (1994) Rediscovery of Chatham Island Tāiko *Pterodroma magentae*. Notornis (Supplement) 41:49-60

Fu Y-X (1994) A phylogenetic estimator of effective population size or mutation rate. Genetics 136:685-692

King M (2000) Moriori A People Rediscovered, 2 edn. Penguin Books (NZ) Ltd, Auckland

Leonard JA, Vila C, Wayne RK (2005) Legacy lost: genetic variability and population size of extirpated US grey wolves *(Canis lupus)*. Molecular Ecology 14:9-17

Matisoo-Smith E, Allen JS, Ladefoged TN, Roberts RM, Lambert DM (1997) Ancient DNA from Polynesian rats: extraction, amplification and sequence from single small bones. Electrophoresis 18:1534-1537

Matocq MD, Villablanca FX (2001) Low genetic diversity in an endangered species: recent or historic pattern? Biological Conservation 98:61-68

Millener PR (1996) Extinct birds. In: The Chatham Islands Heritage and Conservation, pp. 113-120. Canterbury University Press, Christchurch

Millener PR (1999) The history of the Chatham Islands' bird fauna of the last 7000 years - A chronicle of change and extinction. Smithsonian Contributions to Palaeobiology 89:85-109

Miller CR, Waits LP (2003) The history of effective population size and genetic diversity in the Yellowstone grizzly *(Ursus arctos)*: implications for conservation. Proceedings of the National Academy of Sciences 100:4334-4339

Nei M (1987) Molecular Evolutionary Genetics. Columbia University Press, New York

Nunn GB, Cooper J, Jouventin P, Robertson CJR, Robertson GG (1996) Evolutionary relationships among extant albatrosses (Procellariiformes: Diomedeidae) established from complete cytochrome-*B* gene sequences. Auk 113:784-801

Nunn GB, Stanley SE (1998) Body size effects and rates of cytochrome *b* evolution in tube-nosed seabirds. Molecular Biology and Evolution 15:1360-1371

Paxinos EE, James HF, Olson SL, Ballou JD, Leonard JA, Fleischer RC (2002) Prehistoric decline of genetic diversity in the Nene. Science 296:1827

Ritchie PA, Millar CD, Gibb G, Baroni C, Lambert DM (2004) Ancient DNA enables timing of the Pleistocene origin and Holocene expansion of two Adelie Penguin lineages in Antarctica. Molecular Biology and Evolution 21:240-248

Ross JD, Arndt AD, Smith RFC, Johnson JA, Bouzat JL (2006) Re-examination of the historical range of the Greater Prairie Chicken using provenance data and DNA analysis of museum collections. Conservation Genetics 7:735-750

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R (2003) DnaSP, DNA polymorphism analyses by the coalescent and other methods. Bioinformatics 19:2496-2497

Shapiro B, Drummond AJ, Rambaut A, Wilson MC, Matheus PE, Sher AV, Pybus OG, Gilbert MTP, Barnes I, Binladen J, Willerslev E, Hansen AJ, Baryshnikov GF, Burns JA, Davydov S, Driver JC, Froese DG, Harington CR, Keddie G, Kosintsev P, Kunz ML, Martin LD, Stephenson RO, Storer J, Tedford R, Zimov S, Cooper A (2004) Rise and fall of the Beringian steppe bison. Science 306:1561-1565

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555-562

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Science USA 101:15261-15264

Stiller M, Green RE, Ronan M, Simons JF, Du L, He W, Egholm M, Rothberg JM, Keates SG, Ovodov ND, Antipina EE, Baryshnikov GF, Kuzmin YV, Vasilevski AA, Wuenschell GE, Termini J, Hofreiter M, Jaenicke-Despres V, Paabo S (2006) Patterns of nucleotide misincorporations during enzymatic amplification and direct large-scale sequencing of ancient DNA. Proceedings of the National Academy of Science USA 103:13578-13584

Sutton DG (1976) Radiocarbon dates from the Waihora mound site, south-west coast, Chatham Islands. New Zealand Archaeological Association Newsletter 19:195-196

Sutton DG (1979a) Island and coastal fowling strategies of the prehistoric Moriori. In: Birds of a Feather Osteological and Archaeological Papers from the South Pacific in Honour of R. J. Scarlett (ed. Anderson A), pp. 123-139. British Archaeological Reports, Oxford

Sutton DG (1979b) Polynesian Coastal Hunters in the Subantarctic Zone: A Case for the Recognition of Convergent Cultural Adaptation. PhD Thesis, Department of Anthropology. University of Otago, Dunedin

Sutton DG (1980) A culture history of the Chatham Islands. Journal of the Polynesian Society 89:67-93

Sutton DG (1982) The Chatham Islands. In: The First Thousand Years, Regional Perspectives in New Zealand Archaeology (ed. Prickett N), pp. 160-178. Dunmore Press Limited, Palmerston North

Sutton DG (1989) Moriori fishing: intensive exploitation of the inshore zone. In: Saying So Doesn't Make it So (ed. Sutton DG), pp. 116-131. New Zealand Archaeological Association, Dunedin

Tajima F (1996) The amount of DNA polymorphism maintained in a finite population when the neutral mutation rate varies among sites. Genetics 143:1457-1465

Wisely SM, Buskirk SW, Fleming MA, McDonald DB, Ostrander EA (2002) Genetic diversity and fitness in black-footed ferrets before and during a bottleneck. Journal of Heredity 93:231-237

Worthy TH, Holdaway RN (2002) The Lost World of the Moa Prehistoric Life of New Zealand. Canterbury University Press, Christchurch

Wright S (1931) Evolution in Mendelian populations. Genetics 16:97-159

Chapter Four

Detection of the Existence of Undiscovered Individuals of a Critically Endangered Species with Genetic Signatures

Abstract

Many rare and endangered species are difficult to locate, observe and study. As a consequence many individuals, breeding pairs and even populations of such species could remain undetected. Genetic markers can potentially be used to detect undiscovered individuals and/or populations. In many cases such discoveries would be of considerable importance to the successful conservation of a rare and endangered species. Genetic markers were used in an attempt to discover breeding pairs of the Chatham Island Tāiko (Tchāik, *Pterodroma magentae*). The Tāiko is the world's most endangered seabird, with an estimated population size of 120-150 individuals and just 8-15 breeding pairs. The Tāiko is a pelagic gadfly petrel that returns to land only in the breeding season during which it is nocturnal and nests in burrows. In addition, Tāiko burrows are situated in dense forest in a remote area of Chatham Island and are consequently difficult to locate and study. It is important that all Tāiko burrows are discovered to enable monitoring and protection of the birds from predators. In order to examine relationships and thus determine whether all the burrows have been identified, the mitochondrial cytochrome *b* gene and both copies of a fragment of the duplicated domain I of the control region were sequenced. Twenty-one

haplotypes were revealed, including four (19%) not found in birds at known burrows. This suggests there are more burrow groups yet to be located. Furthermore, genetic analyses can aid radio telemetry tracking of Tāiko to burrows by prioritising individuals to track and by recommending specific search areas. The combination of genetic markers together with ecological information and telemetry results has enabled the likely existence of further Tāiko burrows to be detected. This result is of great importance to the conservation of this critically endangered species.

Introduction

It is almost always difficult to locate and study all the individuals of a rare and endangered species. Hence individuals and perhaps groups or even breeding populations of such species can exist undetected. Genetic research provides an important tool for conservation management (Frankham et al. 2002) and can potentially aid the discovery and location of individuals of an endangered species. This possibility is of considerable importance to conservation.

I am unaware of any study using genetic markers to aid in the discovery of unknown breeding individuals or populations and report a novel attempt to do so. The principles used in this research are similar to those of 'genetic tagging' in which natal source populations are identified using genetic markers (e.g. King et al. 2000; Bjorndal et al. 2006). However, in this case individuals were examined on a much smaller scale – burrow of natal origin. This is in part made possible by sampling almost the entire known population. Individuals identified through genetic analysis as having no detected maternal relatives are likely to have an unknown natal location; philopatry could lead them back to that location. Tracking of these individuals could therefore lead to the discovery of further breeding groups.

Current Status of the Chatham Island Tāiko

The Tāiko (Tchāik*, Pterodroma magentae*) is a gadfly petrel endemic to Chatham Island (Rēkohu/Wharekauri) located approximately 860km east of New Zealand (Aikman et al. 2001). Tāiko were abundant until mammalian predatory species were introduced to the Chatham Islands in the early 19th century (Crockett 1994). The Tāiko population size has greatly declined to a currently estimated 120-150 individuals (Scofield 2004). Only 15 Tāiko pairs are known to have bred in recent years, but there are also a larger number of non-breeding individuals (Imber et al. 2005). Breeding occurs in the southwest of Chatham Island,

in and around the Tuku Nature Reserve (Johnston et al. 2003; Fig. 4.1). The Tāiko is ranked 'critically endangered' by the World Conservation Union (IUCN) and New Zealand's Department of Conservation (IUCN 2006; Hitchmough et al. 2007).

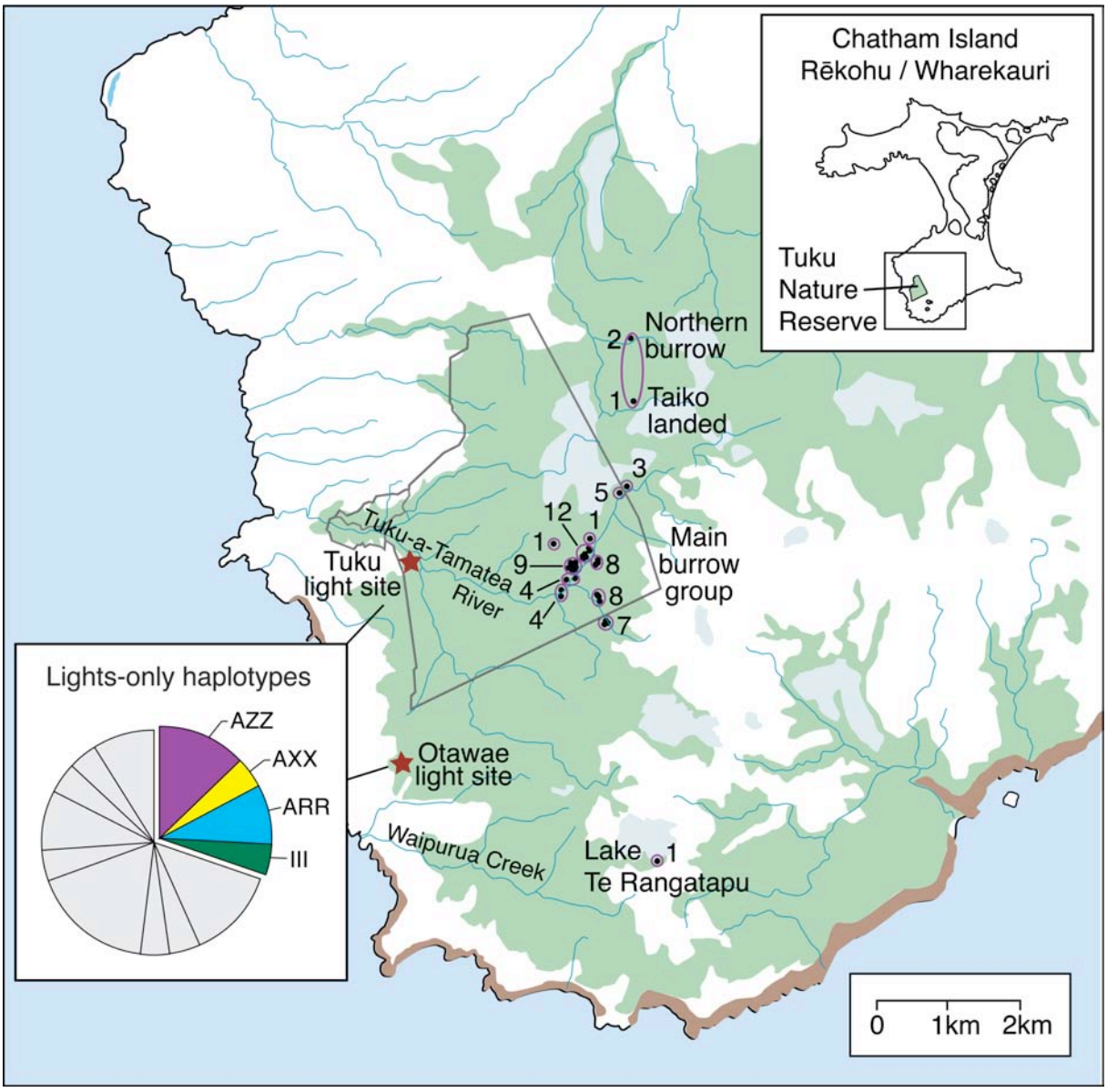

Figure 4.1 The distribution of Tāiko burrows in the southwest of Chatham Island / Rēkohu / Wharekauri. Black dots represent burrows, burrow groups are circled. Numbers of individual Tāiko in the burrow groups are indicated. The pie chart shows mitochondrial DNA haplotypes of Tāiko caught only at the lights, the four haplotypes found only in birds caught at the light sites are identified. These 'lights-only' haplotypes were found in seven individuals.

Difficulties in Studying the Tāiko

Tāiko are challenging to study because they are pelagic, foraging in the open ocean where it is difficult to identify them in flight (Eades and Rogers 1982; Taylor 2000). Tāiko only come to land for a short time in the breeding season during which they make a number of excursions out to sea to feed (Warham 1990; Imber, Taylor et al. 1994; Johnston et al. 2003). When on land, the Tāiko is nocturnal and inhabits underground burrows, making observation extremely difficult (Marchant and Higgins 1990). Tāiko burrows are challenging to find because they are typically in very dense forest at a remote location (Aikman et al. 2001). The rarity of Tāiko and the isolated nature of the burrows add to the difficulty. Furthermore, it can be difficult to determine if burrows are inhabited since they are typically several metres long (Imber, Crockett et al. 1994). Tāiko sexes are effectively indistinguishable except by examination after egg laying or utilisation of molecular techniques (Aikman et al. 2001; chapter five). Consequently, our understanding of the species is limited and there are a number of important management issues relating to the breeding biology of Tāiko that still need to be addressed, such as timing of predator control and chick translocation (Aikman et al. 2001).

Tāiko are thought to be long-lived (perhaps 30-40 years), have delayed maturity and are slow to reproduce (Aikman et al. 2001; Imber et al. 2005). Thus, long-term studies are often required to understand seabird ecology and biology (which can be expensive and require perseverance, Wooller et al. 1992; Moller et al. 2000). These studies have not been possible for Tāiko research, since the Tāiko was considered extinct by Western science until it was rediscovered in 1978. Furthermore, the first active breeding burrow was not found until 1987 (after 17 years of searching, Crockett 1994; Imber, Crockett 1994). Therefore, long-term ecological data are unavailable (not even one cohort of Tāiko has been studied throughout its entire lifespan).

A variety of molecular genetic methods provide extremely useful tools that can reveal life history parameters that are almost impossible to obtain by direct observation (Frankham et al. 2002). The power of genetic techniques to aid species' conservation is increased when used in combination with appropriate ecological studies.

Conservation Initiatives

From a conservation management perspective, Tāiko breeding burrows must be located and monitored in order to protect adults, chicks and eggs from introduced predators. Potential predators found in the vicinity of breeding individuals include feral cats *(Felis catus)*, Australian possums *(Trichosurus vulpecula)*, rats *(Rattus rattus* and *R. exulans)*, feral pigs *(Sus scrofa)* and Weka *(Gallirallus australis*, Taylor 2000). Control of Tāiko predators began

in 1987 (Imber, Taylor et al. 1994) and was intensified from 1996 (Imber et al. 2005). Numbers of fledgling chicks have increased as more breeding burrows are discovered and protected from predators. The location of approximately 15 burrows with potential Tāiko breeding pairs is currently known. Burrows are found by ground searches with the assistance of radio telemetry and using trained dogs (Imber et al. 2005).

Tāiko are caught and tracked approximately biannually using radio telemetry. Spotlighting techniques are used at two 'light sites' to catch Tāiko at night as they fly to or from the colony (Crockett 1994; Imber et al. 2005; Fig. 4.1). Once a Tāiko is caught, a radio-transmitter is attached (Imber, Crockett et al. 1994), the bird is banded and a blood sample is taken. Upon release Tāiko fly out to sea. Tracking stations are used to identify Tāiko individuals returning to land. Tāiko are then followed using hand-held aerials in the forest, with the aim of discovering their nesting burrows (Imber et al. 2005).

The success of radio telemetry in discovering Tāiko burrows has varied over the years. In 1987 radio telemetry led to five burrows being found (including three breeding burrows), the first time seabird burrows have been found using this method (Imber, Crockett et al. 1994). The 1988 season was unsuccessful; telemetry did not occur again until 1993 when two additional breeding burrows were discovered. Two active sites were found in 1997 and at least 25 new burrows in 1999 (Taylor 2000). The discovery of new burrows have continued since then, one notable discovery in 2003 located a burrow much further south than the other known sites, the first in the Waipurua catchments (on the south-east shore of Lake Te Rangatapu, Ballantyne 2004; Fig. 4.1).

Radio telemetry tracking and searching for Tāiko burrows is an expensive and time-consuming process. Analyses of DNA markers such as mitochondrial DNA sequences can be used to assess the likelihood of undiscovered burrows (and therefore justify the effort and expense) and aid the tracking effort by suggesting certain individuals and search areas to target.

Mitochondrial DNA as a Marker

Mitochondrial DNA (mtDNA) is a useful marker because of its relatively high mutation rate (Brown et al. 1979). It is however, haploid and generally maternally inherited (Avise 2004). Different parts of the mitochondrial genome evolve at different rates and for this reason, can provide markers with different levels of genetic variation. The cytochrome *b* gene is involved in the respiratory chain so is relatively conserved (Howell 1989). Despite its limitations in interspecific studies (Meyer 1994) the mitochondrial gene cytochrome *b* can be a useful genetic marker in intraspecific research, if there is sufficient variation (e.g. Cagnon et al.

2004). The control region however, is the fastest evolving region of the mitochondrial genome (Lambert et al. 2002). It consists of three domains with varying mutation rates. Domain I is a useful marker for within-species population genetic studies (Baker and Marshall 1997). Both of these mtDNA regions are commonly used to examine population genetic structure and subdivision. The reasonably high level of mtDNA diversity in the cytochrome *b* gene and domain I of the control region makes them suitable to examine population structure in Tāiko (chapter two).

Methods

Blood samples were collected from Tāiko (*N* = 90) and stored in lysis buffer (Seutin et al. 1991). DNA was extracted using proteinase K digestion and a modified version of the phenol/chloroform method (Sambrook et al. 1989).

The complete mitochondrial cytochrome *b* gene (1143 base pairs, bp) and duplicated segments of domain I of the mitochondrial control region (315 bp each) were sequenced for 78 adult Tāiko and 2 Tāiko chicks (to infer their mothers haplotype, chapter two). For 6 adults only the control region segments were sequenced. The cytochrome *b* haplotype for these 6 individuals and all haplotypes for 4 other adults were inferred from their mother's haplotype. Mothers were identified in the breeding burrow during the season of the chick's birth. In total, haplotypes were identified for the 90 Tāiko adults. GenBank® accession numbers are EU302268-EU302311, EU302313-EU302319, EU302323-EU302324, EU302326-EU302332, EU302334-EU302335, EU302337-EU302374, EU302376, EU302381-EU302395, EU302400-EU302401, EU302403-EU302408, EU302410-EU302411, EU302414-EU302426, EU302428-EU302436, EU302438-EU302440, EU302442-EU302449, EU302454-EU302460. The possible existence of an amplified nuclear pseudogene was tested for and excluded (appendix A). Sequences were aligned using Sequencher™ version 4.2.2 (GeneCodes). A median joining network for cytochrome *b* haplotypes was constructed in Network version 4.201 (Bandelt et al. 1999) and a neighbour joining network for all mtDNA haplotypes was constructed in SplitsTree version 4.6 (Huson and Bryant 2006).

Spatial Autocorrelation Analysis

Spatial autocorrelation analysis uses pair-wise comparisons of the genetic and geographic distances between individuals to detect microgeographic patterns within a population. The autocorrelation coefficient *(r)* measures the genetic similarity between pairs of individuals

whose geographic separation falls within specified geographic distance classes. Many birds exhibit strong natal philopatry and restricted dispersal. These behaviours are likely to result in fine-scale genetic structuring. Spatial autocorrelation analysis was used to test for evidence of fine-scale genetic structuring within Tāiko (using the software GenAlEx version 6, Peakall and Smouse 2006). Initially, a "global" analysis was conducted which included all Tāiko adults that had been either associated with a breeding burrow or a specific geographic location (n = 66). Excluded from the analysis was one male Tāiko that had been associated with multiple locations. The distances between burrows and geographic locations were grouped into distance classes ranging from 10m to 500m; 95% confidence intervals were created using 999 random permutations and 1000 bootstraps. The same analysis was also performed with both sexes separately.

In addition to the global analysis, a "local" analysis was conducted. Local analyses can detect clusters of genetic similarity using subsets of the data (Double et al. 2005). The local autocorrelation of every individual is estimated by comparison to its nearest neighbours. Two-dimensional local spatial autocorrelation analysis (2D LSA) was conducted using the same Tāiko individuals as detailed above (also in GenAlEx version 6, Peakall and Smouse 2006). Both standard and conditional permutations were implemented in order to determine the significance of the local autocorrelation estimates. In standard permutations, all individuals in the subset are randomized among all locations in the dataset. However, in conditional permutations, the pivotal individual is fixed while other individuals in the subset are permutated around all locations. Both types of permutational procedure were tested, with 999 permutations performed for each run. A Bonferroni correction was not applied because such an adjustment is likely to be too conservative (Double et al. 2005). Two to ten nearest neighbours were used for calculation of local spatial autocorrelation. Nearest neighbour distance calculations were also computed in GenAlEx version 6 (Peakall and Smouse 2006).

Results

A large number of haplotypes were detected (21), reflecting a considerable amount of genetic variation within the remnant Tāiko population (chapter two). Of the Tāiko associated with burrows, 5 of these haplotypes were only represented in a single adult each. Phylogenetic analyses did not detect any considerable genetic divergence, except for haplotype C (Fig. 4.2A). The only Tāiko adults with haplotype C were a female born in the Northern burrow

(which moved 3km to the main burrow group and raised at least five fledglings) and a male tracked to the Northern area (Fig. 4.1).

Of the 21 mtDNA haplotypes, four were only from Tāiko caught at light sites and not associated with any burrow (seven birds). These are haplotypes III (one bird), AXX (one bird), ARR (two birds) and AZZ (of which there are three birds). Phylogenetic networks (for example Fig. 4.2B) do not show these 'light-site only' haplotypes clustering together but rather spread throughout the network.

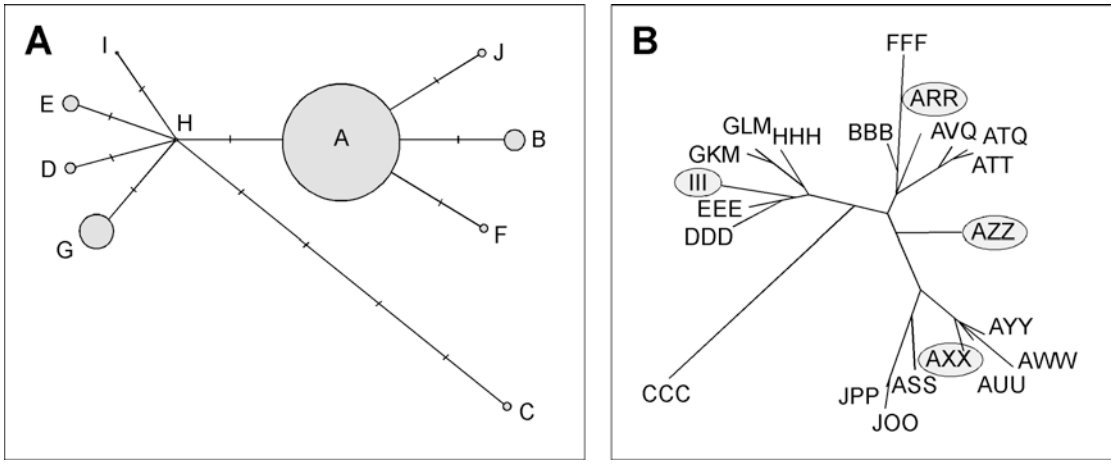

Figure 4.2 Networks of mitochondrial DNA haplotypes of Tāiko **A** Median joining network of cytochrome b haplotypes, ' – ' represents a nucleotide base change between haplotypes, circle size is proportional to number of birds with the haplotype **B** Neighbour joining network of all haplotypes, circles indicate four haplotypes found only in Tāiko caught at the lights and not associated with a geographic location.

Spatial Autocorrelation Analysis

A global analysis detected significant spatial structure among the mtDNA haplotypes of Tāiko i.e. when comparisons were made across the entire adult population for which geographic locations were known *(n* = 66). Global spatial autocorrelation analysis resulted in a significant positive autocorrelation coefficient r-value for the 10m distance class *(P* = 0.026) and 50m distance class *(P* = 0.033, Fig. 4.3). The bootstrap r-value was slightly below the upper 95% confidence limit for both. However, bootstrap tests are more conservative than permutation tests when the sample size is small (Temple et al. 2006). Significant global spatial structure was not detected when the sexes were analysed separately.

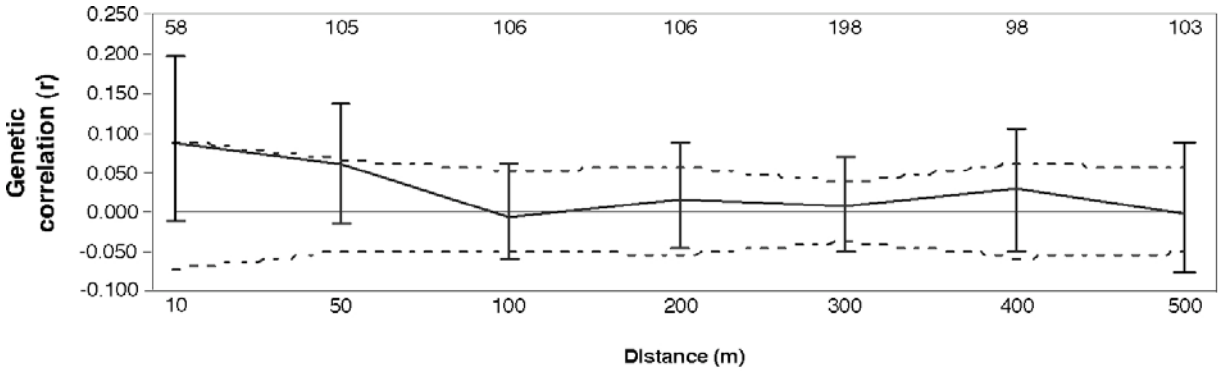

Figure 4.3 Correlogram plot of the genetic correlation coefficient *(r)* as a function of distance (m) for adult Tāiko associated with known locations *(n* = 66). Dashed lines represent the permuted 95% confidence intervals; bars represent bootstrapped 95% confidence error. Numbers above data points indicate number of pairwise comparisons within each distance class.

Global spatial autocorrelation analysis does not describe the relationships between different geographical groups or individuals. Clusters of genetic autocorrelation can exist even when there is little or no significant global spatial structure (Double et al. 2005). Therefore, a local spatial autocorrelation analysis was implemented. This analysis requires specification of the number of individuals to be regarded as 'nearest-neighbours' to compare to a pivotal individual. Nearest neighbour distance calculations suggested that four nearest neighbours was the most appropriate parameter since the mean distance is 58m (when four outliers are excluded, see Fig. 4.4), which typically included birds within the same burrow group (Table 4.1). Burrow groups are units that have been arbitrarily defined based on geographic proximity. Local correlation values (*lr*, for four nearest neighbours, 4NN) were significantly positive *(P* < 0.05) for 26% of the 66 adult Tāiko (of both sexes) in a one-tailed test using conditional permutations. The locations of these individuals showed there were three 'hotspots' where a burrow group included four individuals with significant *lr* (Fig. 4.4). Standard and conditional permutations produced similar results over the range of 1 to 10 nearest neighbours, although slightly more *lr* values were significant when conditional permutations were implemented. The number of significant *lr* values ranged from 8 to 17 when conditional permutations were used (for 2 to 10 nearest neighbours) and 7 to 15 when standard permutations were used (Table 4.1). The pattern was therefore consistent over the 2 to 10 nearest neighbour classes.

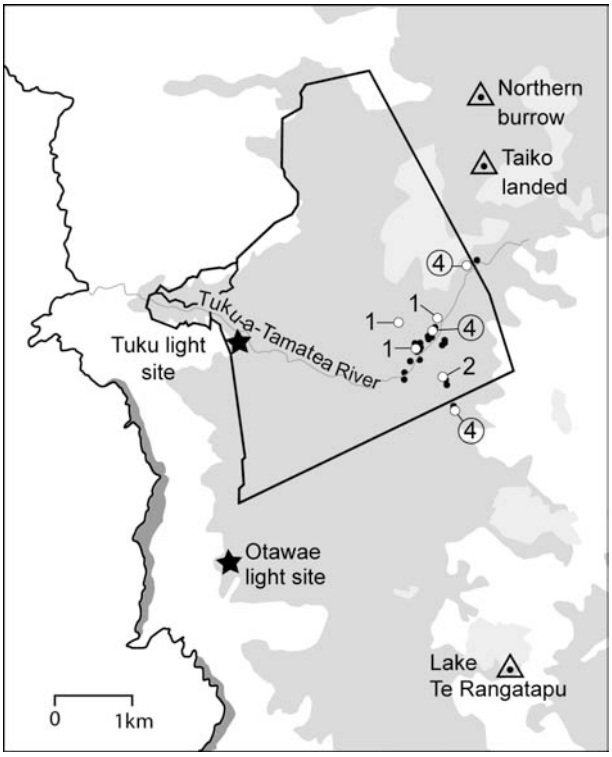

Figure 4.4 The distribution of Tāiko with significant local correlation values *(lr*, white dots), identified by two-dimensional local spatial autocorrelation analysis of adults associated with a known location *(n* = 66, for four nearest neighbours). Numerals indicate number of individuals *(n* = 17). Significant *lr* values range from 0.07 to 0.66. Black dots represent other burrow locations. The four outliers excluded from nearest neighbour distance calculations are indicated by triangles (i.e. the individual in the burrow by Lake Te Rangatapu, the Tāiko that landed in the northern area, and the two birds from the northern burrow). 'Hotspots' containing four individuals with significant *lr* are circled.

Table 4.1 Results of a two-dimensional local spatial autocorrelation analysis in Tāiko (using a one-tailed test with conditional permutations in GenAlEx version 6).

Number of nearest neighbours	1	2	3	4 [a]	5	6	7	8	9	10
Mean geographic distance (m) [b]	11	15	37	58	78	88	124	260	303	314
Number of individuals with significant [c] *lr* [d] values										
All adults (*n* = 66)	3	11	14	17	10	8	11	15	11	11
Males (*n* = 40)	0	3	6	5	5	8	8	7	4	7
Females (*n* = 26)	1	2	2	1	0	0	0	0	0	0

[a] *Shaded column highlights values determined using four nearest neighbours*
[b] *For calculation of mean geographic distance (m = metres) four outliers in northern and southern areas were excluded (see Fig. 4.4)*
[c] *Significant at P < 0.05*
[d] *lr = local correlation values*

For 40 adult Tāiko males, 13% had significant local correlation values *(lr)* in a one-tailed test using conditional permutations *(P* < 0.05, for 4NN). Three to eight *lr* values were significant over the range of two to ten nearest neighbours (Table 4.1). In contrast, for the 26 adult Tāiko females, very few had significant *lr* values. For two and three nearest neighbours there were two significant *lr* values, for five through to ten nearest neighbours there were no significant *lr* values (using a one-tailed test with conditional permutations, Table 4.1). Although the data set was small (*n* = 26), results suggested very little or no spatial genetic structure for females.

Discovery of Burrow Groups

Tracking Tāiko with mtDNA haplotypes not found in any birds at known burrows can lead to the discovery of new burrow groups. There is an example of this when the haplotypes of Taiko that in the past have led to the discovery of breeding burrows are retrospectively examined. Previously, a mtDNA haplotype was represented only in two Tāiko, both caught at the light site and not associated with a geographic location. One of these Tāiko (a male) was tracked to a new burrow, and four others with the same haplotype were subsequently found in the same area. The tracking of the male Tāiko with a 'lights-only' haplotype led to the discovery of a large number of other burrows and the direct capture of seven previously unknown Tāiko. At that time the mtDNA haplotypes were not known, but this provides an example that the method using genetic signatures can lead to the discovery of new burrow groups.

Discussion

Philopatry in Tāiko

The determination of the mtDNA haplotypes of Tāiko caught at the light sites can assist in the location of new burrows by recommending specific birds to track and by suggesting geographic locations to search. The success of this management tool relies on sufficient genetic variation and on Tāiko exhibiting strong philopatric behaviour. If birds generally return to breed near their natal burrow, they should return near birds with the same mtDNA haplotype. Spatial autocorrelation analysis of mtDNA haplotypes detected significant global genetic structure for distance classes of 10m and 50m, which suggested philopatry and limited dispersal from the natal burrow site.

It is thought that males are typically the more philopatric sex in birds (Clarke et al. 1997) and studies of petrels are consistent with this difference between the sexes (e.g. Mougin et al.

1999). The results of local spatial autocorrelation analysis suggested that male Tāiko are more philopatric than females. However, more significant local correlation was apparent when sexes were combined than when they were separated. This could be due to the maternal inheritance of mitochondrial DNA, i.e. males may be located near their mother, a situation that is undetected when females are excluded from analysis. The level of significant local correlation values for Tāiko adults of both sexes (26% for 4NN) was similar to that found in philopatric male Fairy-Wrens (Double et al. 2005). The phenomenon of local genetic correlation 'hotspots' previously observed in Fairy-Wrens (Double et al. 2005) were also apparent in Tāiko (Fig. 4.4). These 'hotspots' may be caused by the return of philopatric offspring of relatively highly productive parents.

The local spatial autocorrelation analysis of mtDNA data for Tāiko suggested philopatry despite small sample size and the inclusion of individuals from a long time period (a decade). In the future, genetic analysis of a larger sample size over a shorter time period could suggest a stronger philopatric trend as greater numbers of fledglings produced more recently (since predator control began) return as adults. In addition, mark-recapture data indicate that a total of nine Tāiko initially caught as chicks have returned to burrows in the colony. Examination of movement of the nine re-captured birds from their natal burrows also supports philopatry to the natal area. Five of the birds inhabited burrows adjacent to their natal burrow, two were found in burrows in the adjacent burrow group. Interestingly, the two returning chicks that did not show philopatry were females whereas all others were male. Another two Tāiko first caught as chicks, recaptured at the light site and not found on land, were also females.

Probable Existence of More Burrow Groups

Of 21 mtDNA haplotypes, four (19%) were represented only in Tāiko caught at the light sites and not associated with known burrow locations. This indicated there are more burrows that remain undiscovered. However, these haplotypes did not form a separate cluster in phylogenetic networks (Fig. 4.2), perhaps suggesting that these undiscovered burrows form part of the existing colony rather than a genetically isolated colony, for example, on another island.

One area in which Tāiko could remain undetected is the Waipurua catchment (Fig. 4.1). It is relatively unexplored, yet contains suitable habitat for Tāiko. A male Tāiko was tracked to this area but a burrow was not discovered. This bird has a haplotype only found in Tāiko caught at the light sites, which provides evidence for the Waipurua as a likely location for undiscovered burrows.

From 1978 to 2005, 62 Tāiko have been caught at lights and not subsequently found on land. Of these birds, 63% have not been blood sampled (because they were caught prior to 1996 when sampling began). In total, 54 Tāiko were first caught over a decade ago. Of these birds, 41 (76%) have not been subsequently caught on land, whereas 13 (24%) have. Of the recaptured birds, 5 were not seen for many years (14-21 years), which suggests they were breeding in unknown areas during this time. The other 41 'old' birds may also be breeding in unknown areas. These numbers further support the suggestion that there are Tāiko breeding in burrows yet to be found.

Conclusions

Data obtained in this study were consistent with the Tāiko having philopatric tendencies and this can be used to aid the search for further individuals inhabiting unknown burrows and/or burrow groups. Mitochondrial DNA data suggested the probable existence of unknown burrow groups, with 19% of haplotypes identified in birds not associated with a known burrow. The mtDNA data identified individual Tāiko that would be of high priority for tracking and could narrow the search area for other birds. The discovery of further Tāiko is important to enable protection from predators and is essential for the long-term survival of the species.

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References

Aikman H, Davis A, Miskelly C, O'Conner S, Taylor GA (2001) Chatham Island Tāiko Recovery Plan 2001-2011. Threatened Species Recovery Plan 36. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/TSRP36.pdf

Avise JC (2004) Molecular Markers, Natural History, and Evolution, 2 edn. Sinauer Associates, Massachusetts

Baker AJ, Marshall HD (1997) Mitochondrial control region sequences as tools for understanding evolution. In: Avian Molecular Evolution and Systematics (ed. Mindell DP), pp. 51-82. Academic Press, Ann Arbor

Ballantyne J (2004) Tāiko Telemetry Operation- 2003. Department of Conservation, Wellington

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37-48

Bjorndal KA, Bolten AB, Moreira L, Bellini C, Marcovaldi MA (2006) Population structure and diversity of Brazilian green turtle rookeries based on mitochondrial DNA sequences. Chelonian Conservation and Biology 5:262-268

Brown WM, George MJ, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Science USA 76:1967-1971

Cagnon C, Lauga B, Hemery G, Mouches C (2004) Phylogeographic differentiation of Storm Petrels *(Hydrobates pelagicus)* based on cytochrome *b* mitochondrial DNA variation. Marine Biology 145:1257-1264

Clarke AL, Saether B-E, Roskaft E (1997) Sex biases in avian dispersal: a reappraisal. Oikos 79:429-438

Crockett DE (1994) Rediscovery of Chatham Island Tāiko *Pterodroma magentae*. Notornis (Supplement) 41:49-60

Double M, Peakall R, Beck N, Cockburn A (2005) Dispersal, philopatry, and infidelity: dissecting local genetic structure in Superb Fairy-Wrens *(Malurus cyaneus).* Evolution 59:625-635

Eades DW, Rogers AEF (1982) Comments on the identification of the Magenta Petrel and similar species. Notornis 29:81-84

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Conservation Genetics. Cambridge University Press, Cambridge

Hitchmough R, Bull L, Cromarty P (comps) (2007) New Zealand Threat Classification System Lists 2005. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/sap236.pdf

Howell N (1989) Evolutionary conservation of protein regions in the protonmotive cytochrome *b* and their possible roles in redox catalysis. Journal of Molecular Evolution 29:157-169

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23:254-267

Imber MJ, Crockett DE, Gordon AH, Best HA, Douglas ME, Cotter RN (1994) Finding the burrows of Chatham Island Tāiko *Pterodroma magentae* by radio telemetry. Notornis (Supplement) 41:69-96

Imber MJ, Taylor GA, Grant AD, Munn A (1994) Chatham Island Tāiko *Pterodroma magentae* management and research, 1987-1993: predator control, productivity, and breeding biology. Notornis (Supplement) 41:61-68

Imber MJ, Taylor GA, Tennyson AJD, Aikman HA, Scofield RP, Ballantyne J, Crockett DE (2005) Non-breeding behaviour of Magenta Petrels *Pterodroma magentae* at Chatham Island, New Zealand. Ibis 147:758-763

IUCN (World Conservation Union) (2006) IUCN Red List of Threatened Species 2006. IUCN, Gland, Switzerland. Available from http://www.iucnredlist.org (accessed June 2007)

Johnston RB, Bettany SM, Ogle RM, Aikman HA, Taylor GA, Imber MJ (2003) Breeding and fledging behaviour of the Chatham Tāiko (Magenta Petrel) *Pterodroma magentae* and predator activity at burrows. Marine Ornithology 31:193-197

King TM, Williams M, Lambert DM (2000) Dams, ducks and DNA: identifying the effects of a hydro-electric scheme on New Zealand's endangered Blue Duck. Conservation Genetics 1:103-113

Lambert DM, Ritchie PA, Millar CD, Holland B, Drummond AJ, Baroni C (2002) Rates of evolution in ancient DNA from Adelie Penguins. Science 295:2270-2273

Marchant S, Higgins PJ (1990) Handbook of Australian, New Zealand and Antarctic birds. Oxford University Press, Melbourne

Meyer A (1994) Shortcomings of the cytochrome *b* gene as a molecular marker. Trends in Ecology and Evolution 9:278-280

Moller H, Frampton C, Hocken AG, McLean IG, Saffer V, Sheridan L (2000) The importance of seabird research for New Zealand. New Zealand Journal of Zoology 27:255-260

Mougin J-L, Granadeiro JP, Jouanin C, Roux F (1999) Philopatry and faithfulness to nest site in Cory's Shearwaters *Calonectris diomedea* at Selvagem Grande. Ostrich 70:229-232

Peakall R, Smouse P (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York

Scofield RP (2004) Population assessment of the endangered Chatham Island Tāiko. In: Third International Albatross and Petrel Conference, Montevideo, Uruguay

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82-90

Taylor GA (2000) Action Plan for Seabird Conservation in New Zealand Part A: Threatened Seabirds. Threatened Species Occasional Publication 16. Department of Conservation, Wellington http://www.doc.govt.nz/upload/documents/science-and-technical/TSOP16.pdf

Temple HJ, Hoffman JI, Amos W (2006) Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the White-breasted Thrasher, *Ramphocinclus brachyurus*. Molecular Ecology 15:3449-3458

Warham J (1990) The Petrels Their Ecology and Breeding Systems. Academic Press, London

Wooller RD, Bradley JS, Croxall JP (1992) Long-term population studies of seabirds. Trends in Ecology and Evolution 7:111-114

Chapter Five

Excess of Unpaired Males in the World's Most Endangered Seabird

Abstract

The Chatham Island Tāiko (Tchāik, *Pterodroma magentae*) is the world's most endangered seabird with a population size of between 120-150 individuals that includes only 8-15 breeding pairs. Molecular techniques were used to identify the sex of Tāiko, which is difficult to assign morphologically. Blood samples were obtained from almost the entire known living population and from some birds now thought to be dead. An approximately even sex ratio was found in Tāiko chicks and adults associated with breeding burrows, but a large male-biased ratio was identified in non-breeding adult birds caught on the ground. This finding suggests that unpaired males may be having difficulty in attracting females to burrows and that this situation may be an example of the Allee effect, that reduced density of potential mates is acting to decrease population productivity. Identification of the sex of Tāiko using a molecular technique has important implications for the conservation management of this critically endangered species, including the future transfer of Tāiko chicks to a predator-excluded breeding site.

Introduction

The world's most endangered seabird is the Chatham Island Tāiko (Tchāik, *Pterodroma magentae*), ranked critically endangered by the World Conservation Union, IUCN, and New Zealand's Department of Conservation (IUCN 2006; Hitchmough et al. 2007). There are only approximately 120 to 150 Tāiko (Scofield 2004) and the species is vulnerable to predation by exotic species (Taylor 2000; Johnston et al. 2003). The only known Tāiko population exists as a single colony on the main Chatham Island (Rēkohu/Wharekauri), which is located around 860km east of the South Island of New Zealand (Aikman 2001; Fig. 5.1A). Tāiko nest 4 to 5km inland in very scattered burrows (which are often solitary) in dense forest (Fig. 5.1B, C). Only 15 Tāiko pairs are known to have bred in recent years, but there are also larger numbers of non-breeding individuals (Imber et al. 2005). The average age of first reproduction in Tāiko is seven to nine years. Determining the sex ratio in non-breeding birds is crucial to understanding whether these individuals are unpaired because of a lack of potential mates or other causes. The sex ratio of Tāiko chicks fledged is also important for future management of the colony.

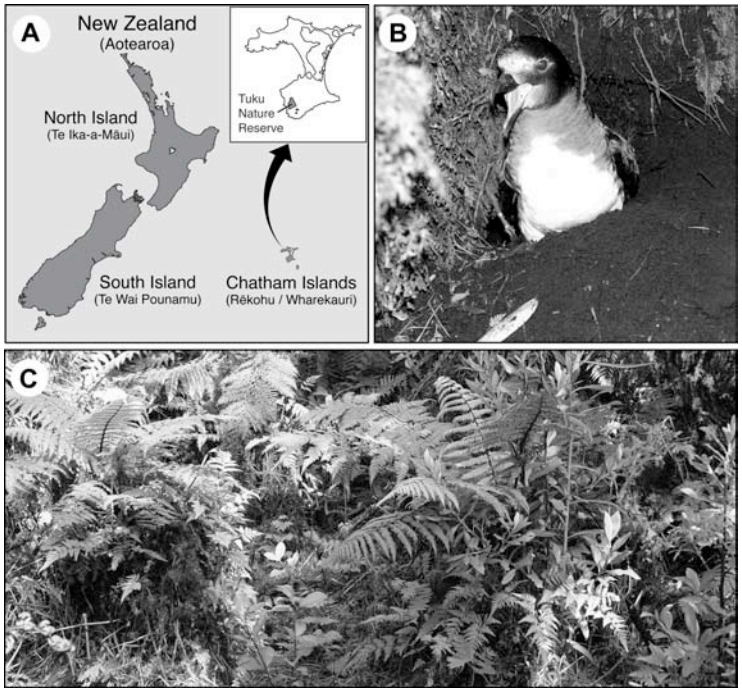

Figure 5.1 The geographic location, burrows and breeding habitat of the Tāiko. A) New Zealand / Aotearoa and the Chatham Islands / Rēkohu / Wharekauri B) a male Tāiko calling from the entrance of its burrow C) typical breeding habitat of Tāiko. Photographs by Graeme A. Taylor.

Fledgling survival is critical in order to prevent extinction of the Tāiko. Tāiko chicks are vulnerable to predation so intense predator control is maintained around burrows and fledgling flight paths during the breeding season (Aikman et al. 2001). A predator exclusion fence has been built around the site of an extinct Tāiko colony (Stephenson 2006). Management plans include the translocation of Tāiko chicks to artificial burrows within this safer area (Aikman et al. 2001). Such a transfer would occur before chicks emerge from burrows to increase the likelihood that they will become imprinted on the new site (as with other petrels, Gummer 2003; Miskelly and Taylor 2004; Bell et al. 2005; Priddel et al. 2006). It is anticipated that the birds' philopatric tendencies will result in Tāiko returning to the predator-excluded area after they return from the sea aged four to six years old (Imber et al. 2005). Males are thought to be more philopatric than females in birds generally (Greenwood 1980; Clarke et al. 1997); examples in petrels include the Manx Shearwater *(Puffinus puffinus*, Brooke 1978), Cory's Shearwater *(Calonectris diomedea*, Mougin et al. 1999) and Wandering Albatross *(Diomedea exulans*, Weimerskirch et al. 2005). In Tāiko it is the males that choose and dig the nest site (Imber et al. 2005; Fig. 5.1B). Male chicks will therefore be preferentially selected for translocation in the future, since they may be more likely to return to the new colony (chapter three). However, transfer of Tāiko chicks in the short-term will initially include both sexes to build a critical mass of birds. The identification of the sex of chicks is important before they are transferred. Tāiko will also be attracted to the new colony from flight by broadcasting of vocalisations (Taylor 2000; Aikman et al. 2001). Vocal attraction techniques have been successfully used in colony establishment of other petrels *(Oceanodroma leucorhoa,* Podolsky and Kress 1989; *Pelecanoides urinatrix*, Miskelly and Taylor 2004; *Puffinus gavia*, Bell et al. 2005) and are a proven lure for *Pterodroma* (Podolsky and Kress 1992).

Many birds including *Pterodroma* species (Marchant and Higgins 1990) are sexually monomorphic, so genetic sexing techniques have been developed (e.g. Griffiths et al. 1998). DNA sexing techniques have been particularly useful in the conservation of endangered species (e.g. Groombridge et al. 2004). Polymerase chain reaction (PCR) primers are used here to routinely determine the sex of Tāiko adults and chicks.

Methods

Over a 20-year period, field parties have spent hundreds of hours searching for burrows and observing known burrow sites at night in order to catch Tāiko. Tāiko were also caught in flight

using spotlighting techniques (Crockett 1994) and by "war-whooping" (human calls that attract petrels, Tennyson and Taylor 1990). In addition, Tāiko were observed using infrared time-lapse video monitoring. Blood samples were collected from almost all known Tāiko (caught since October 1996, *N* = 148) and stored in lysis buffer (Seutin et al. 1991). High molecular weight DNA was extracted by proteinase K digestion and a modified version of the phenol/chloroform method (Sambrook et al. 1989). Breast feather samples were taken from one Tāiko; genomic DNA was extracted from the tip of the feather using a method adapted from Walsh et al. (1991).

DNA sexing of Tāiko involved the use of the *c*hromodomain-*h*elicase-*D*NA *(CHD)* gene. Primers P2 (Griffiths and Tiwari 1995) and P8 (Griffiths et al. 1998) were used in a PCR modified from that described by Griffiths et al. (1998). Amplified PCR products were digested with the restriction enzyme *Hae III* (Invitrogen), then size fractionated on 2% agarose gels stained with ethidium bromide and visualised under UV light. Divergence from a 1:1 sex ratio of males to females was tested using two-tailed exact binomial tests of goodness of fit (in Microsoft Excel).

Results and Discussion

DNA Sexing Techniques

No inconsistencies were detected in the sexing of 148 Tāiko using the *CHD* method. The amplified fragment of the W (female) chromosome was approximately 390 base pairs (bp) in size and the band size of the Z chromosome fragment (found in both sexes) was 325bp, after digestion. Two other independent sexing tests based on DNA hybridisation were also used and the sex of all 22 Tāiko analysed using these methods were consistent with the *CHD* results (C. D. Millar unpubl. data). Mis-assignment of sex using the *CHD* method has been reported in a congeneric species *Pterodroma arminjoniana* (Ruth M. Brown unpubl. data cited in Robertson and Gemmell 2006). However, in contrast to Robertson and Gemmell's suggestion it now appears that human rather than methodological error was the cause of sex mis-assignment in this species (Ruth M. Brown pers. comm.).

Tāiko Sex Ratios

The sex ratio in the Tāiko chicks is approximately even, as is that of the adults associated with breeding burrows (tested using two-tailed exact binomial tests of goodness of fit, Table 5.1). Tāiko caught from flight using spotlighting techniques (Crockett 1994) were male-biased, but

this difference is not significant *(P* = 0.10). Males make more flights inland than females and are therefore more likely to be caught at the light site (Imber et al. 2005). However, the non-breeding birds caught on the ground have a disparate sex ratio with significantly more males than females (Table 5.1). In addition, all Tāiko landing from flight in response to "war-whooping" (three individuals) or found calling from the ground, have been males. In contrast, in Grey-faced Petrels (Oi, *Pterodroma macroptera gouldi*), a close relative of Tāiko, it is predominantly females that land in response to human calling (G. A. Taylor pers. comm.). Furthermore, non-breeding Tāiko males have been caught on the ground or found in burrows without being attracted by calls and have been observed using infrared video. In contrast, no females have ever been found on the surface at night (except two birds tracked to burrows using radio transmitters). Again this is in stark contrast to the non-threatened Grey-faced Petrel. Over twice as many female Grey-faced Petrels as males were caught on the surface during courtship and incubation periods *(N* = 694, G. A. Taylor unpubl. data).

Table 5.1 Sex ratios in Tāiko *(Pterodroma magentae)*

	Males	**Females**	**TOTAL**	***P*-value**
Chicks (since 1993)	32	35	67	0.81
Adults in total	54	37	91	0.09
Adults caught by spotlighting (since 1996)	28	16	44	0.10
Adults associated with breeding burrows	23	22	45	1
Adults caught at non-breeding burrows or on the surface, not associated with breeding burrows	22	1	23	<0.001 (5.7×10^{-6})

Note: There is overlap in the above ratios: 21 adults caught by spotlighting have also been captured on land, 10 Tāiko caught as chicks have since been captured as adults. Therefore, 27 individuals are represented twice and four individuals are represented three times (i.e. they were caught as chicks, as adults by spotlighting and as adults on the ground).

Although there is a male bias in non-breeding birds, there is not a significant excess of males in the adult population as a whole *(P* = 0.09). Therefore, the disparate sex ratio found in non-breeding Tāiko caught on the ground could be due to difficulties in males attracting mates

to land. The sex ratio of birds caught in flight using spotlights is not significantly male biased and is therefore consistent with this hypothesis. Male Tāiko attract mates by calling from the ground and through aerial courtship (Imber et al. 2005; G. A. Taylor pers. comm.). Non-breeding males have been observed (on infra-red video) calling from burrows, but no female has ever been seen entering these burrows or landing nearby in response (despite hundreds of hours of time-lapse footage, G. A. Taylor pers. comm.). The age at first breeding also suggests that males may have difficulty in attracting mates (although the sample size is very small). One male has bred at age seven and another at age eight years. However, there is one male still not breeding that is at least 9 years of age, three males at least 11 years old and two males aged at least 12. One Tāiko male did not breed until at least 16 years old. Conversely, most females are new breeders in the first season they are detected in a burrow. Of known age birds, one female bred at age seven and another aged nine (G. A. Taylor unpubl. data).

Conservation Management Implications

The extraordinary number of unpaired Tāiko males revealed by molecular sexing is suggestive of the Allee effect (Allee et al. 1949; Berec et al. 2006). This effect maintains that reduced density of conspecifics in the population acts to decrease the population productivity (Courchamp et al. 1999). In Tāiko, when the population size was reduced, burrows became less concentrated thereby decreasing the density of individuals. This might make it more difficult for males and females to locate each other for mating and may explain the high number of unpaired males. Consequently, the risk of species extinction is likely to be increased by the Allee effect (Stephens and Sutherland 1999) and so should be of major concern to Tāiko conservation.

The initiative to establish a new Tāiko colony plans to overcome this effect by creating a higher density of birds within a 4ha area. It is well known that petrels usually form dense nesting colonies (Marchant and Higgins 1990). This higher density of birds could be achieved through Tāiko translocation and vocal attraction techniques. An essential aspect of this scheme is that the new Tāiko colony will be protected from predators by an exclusion fence (Aikman et al. 2001).

Establishment of the new colony involves translocation of Tāiko chicks. It is important that the sex of the chicks is identified before transfer. Slightly more male chicks could be preferentially selected for translocation, as they are more likely to return to the new colony due to their stronger philopatric tendencies (chapter three). It is thought that male seabirds are more philopatric generally; examples of this in petrels includes the Manx Shearwater

(Puffinus puffinus, Brooke 1978), Cory's Shearwater *(Calonectris diomedea*, Mougin et al. 1999) and Wandering Albatross *(Diomedea exulans*, Weimerskirch et al. 2005). Also, in Tāiko it is the males that choose and dig the nest site (Imber et al. 2005). Females may be more likely to be attracted from flight (as in Grey-faced Petrels, G. A. Taylor pers. comm.) and therefore could even the sex ratio in the new colony. Transfer of Tāiko chicks in the short-term however, will initially include both sexes to build a critical mass of birds. DNA sexing provides the only technique to reliably identify sex in Tāiko chicks and therefore will be important in this conservation management initiative.

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References

Aikman H, Davis A, Miskelly C, O'Conner S, Taylor GA (2001) Chatham Island Tāiko Recovery Plan 2001-2011. Threatened Species Recovery Plan 36. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/TSRP36.pdf

Allee WC, Park O, Emerson AE, Park T, Schmidt KP (1949) Principles of Animal Ecology. Saunders, Philadelphia

Bell M, Bell BD, Bell EA (2005) Translocation of Fluttering Shearwater *(Puffinus gavia)* chicks to create a new colony. Notornis 52:11-15

Berec L, Angulo E, Courchamp F (2006) Multiple Allee effects and population management. Trends in Ecology and Evolution 22:185-191

Brooke M de L (1978) The dispersal of female Manx Shearwaters. Ibis 120:546-551

Clarke AL, Saether B-E, Roskaft E (1997) Sex biases in avian dispersal: a reappraisal. Oikos 79:429-438

Courchamp F, Clutton-Brock T, Grenfell B (1999) Inverse density dependence and the Allee effect. Trends in Ecology and Evolution 14:405-410

Crockett DE (1994) Rediscovery of Chatham Island Tāiko *Pterodroma magentae*. Notornis (Supplement) 41:49-60

Greenwood PJ (1980) Mating systems, philopatry, and dispersal in birds and mammals. Animal Behaviour 28:1140-1162

Griffiths R, Double MC, Orr K, Dawson RJG (1998) A DNA test to sex most birds. Molecular Ecology 7:1071-1075

Griffiths R, Tiwari B (1995) Sex of the last wild Spix's Macaw. Nature 375:454

Groombridge JJ, Massey JG, Bruch JC, Malcom T, Brosius CN, Okada MM, Sparklin B, Fretz JS, VanderWerf EA (2004) An attempt to recover the Po'ouli by translocation and an appraisal of recovery strategy for bird species of extreme rarity. Biological Conservation 118:365-375

Gummer H (2003) Chick translocation as a method of establishing new surface-nesting seabird colonies: a review. DOC Science Internal Series 150. Department of Conservation, Wellington http://www.doc.govt.nz/upload/documents/science-and-technical/dsis150.pdf

Hitchmough R, Bull L, Cromarty P (comps) (2007) New Zealand Threat Classification System Lists 2005. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/sap236.pdf

Imber MJ, Taylor GA, Tennyson AJD, Aikman HA, Scofield RP, Ballantyne J, Crockett DE (2005) Non-breeding behaviour of Magenta Petrels *Pterodroma magentae* at Chatham Island, New Zealand. Ibis 147:758-763

IUCN (World Conservation Union) (2006) IUCN Red List of Threatened Species 2006. IUCN, Gland, Switzerland. Available from http://www.iucnredlist.org (accessed June 2007)

Johnston RB, Bettany SM, Ogle RM, Aikman HA, Taylor GA, Imber MJ (2003) Breeding and fledging behaviour of the Chatham Tāiko (Magenta Petrel) *Pterodroma magentae* and predator activity at burrows. Marine Ornithology 31:193-197

Marchant S, Higgins PJ (1990) Handbook of Australian, New Zealand and Antarctic Birds. Oxford University Press, Melbourne

Miskelly CM, Taylor GA (2004) Establishment of a colony of Common Diving Petrels *(Pelecanoides urinatrix)* by chick transfers and acoustic attraction. Emu 104:205-211

Mougin J-L, Granadeiro JP, Jouanin C, Roux F (1999) Philopatry and faithfulness to nest site in Cory's Shearwaters *Calonectris diomedea* at Selvagem Grande. Ostrich 70:229-232

Podolsky RH, Kress SW (1989) Factors affecting colony formation in Leach's Storm-Petrel. Auk 106:332-336

Podolsky RH, Kress SW (1992) Attraction of the endangered Dark-rumped Petrel to recorded vocalizations in the Galapagos Islands. Condor 94:448-453

Priddel D, Carlile N, Wheeler R (2006) Establishment of a new breeding colony of Gould's Petrel *(Pterodroma leucoptera leucoptera)* through the creation of artificial nesting habitat and the translocation of nestlings. Biological Conservation 128:553-563

Robertson BC, Gemmell NJ (2006) PCR-based sexing in conservation biology: wrong answers from an accurate methodology? Conservation Genetics 7:267-271

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York

Scofield RP (2004) Population assessment of the endangered Chatham Island Tāiko. In: Third International Albatross and Petrel Conference, Montevideo, Uruguay

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82-90

Stephens PA, Sutherland WJ (1999) Consequences of the Allee effect for behaviour, ecology and conservation. Trends in Ecology and Evolution 14:401-405

Stephenson B (2006) Petrel head. Forest and Bird Magazine 321:32-33

Taylor GA (2000) Action Plan for Seabird Conservation in New Zealand Part A: Threatened Seabirds. Threatened Species Occasional Publication 16. Department of Conservation, Wellington http://www.doc.govt.nz/upload/documents/science-and-technical/TSOP16.pdf

Tennyson AJD, Taylor GA (1990) Behaviour of *Pterodroma* petrels in response to "War-Whoops". Notornis 37:121-128

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506-513

Weimerskirch H, Lallemand J, Martin J (2005) Population sex ratio variation in a monogamous long-lived bird, the Wandering Albatross. Journal of Animal Ecology 74:285-291

Chapter Six

Nuclear Genetic Data Illuminates Behaviour and Familial Relationships in Tāiko

Abstract

Birds exhibit a range of mating systems including monogamy, communal breeding and promiscuous breeding, the latter includes lekking species. Monogamy is however, the most common mating system in avian species. Monogamous breeding systems have been assumed to represent exclusive mating relationships, however genetic studies have revealed significant levels of extra-pair paternity and egg dumping in many apparently monogamous species. These behaviours have important implications for the conservation of endangered species. The Chatham Island Tāiko (Tchāik, *Pterodroma magentae*) is an extremely rare pelagic seabird, coming to land only during the breeding season when it is nocturnal and inhabits underground burrows. To examine the mating system, behaviour and genealogical relationships in the Chatham Island Tāiko population, individuals were genotyped for eight microsatellite DNA loci. Knowledge of relationships gained from parentage, sibship and fine-scale genetic structure analyses allowed understanding of mating systems and behaviour such as philopatry. Genetic results signified the presence of undiscovered Tāiko and could aid in the search for these individuals.

Introduction

Endangered species are typically found in small numbers, potentially allowing a complete analysis of the genealogical relationships of individuals in the population to be possible. Knowledge of these relationships can provide an understanding of the mating system, behaviour and demography that is important for conservation planning and management (Wayne and Morin 2004). Genetic studies are strengthened when detailed behavioural, biological and ecological data are also available for the study species. Conversely, genetic research is invaluable for species that are logistically difficult to study due their remote location and/ or life history characteristics. Collaboration between field ecologists and molecular biologists is therefore essential to gain the most out of both types of data (Gowaty and Gibbs 1993).

The Chatham Island Tāiko (Tchāik, *Pterodroma magentae*) is a gadfly petrel endemic to the main Chatham Island (Rēkohu/Wharekauri, Aikman et al. 2001). The Tāiko was considered extinct by Western science until 1978 and is now ranked "critically endangered" (Crockett 1994; IUCN 2006; Hitchmough et al. 2007). Presently there are only 15 Tāiko pairs known to have bred in recent years. In addition, there are a number of non-breeding individuals (Imber et al. 2005) and the entire species is thought to comprise approximately 120-150 birds (Scofield 2004). Some life history characteristics of Tāiko that are known are shared with other seabird species (Schreiber and Burger 2002). These include delayed breeding (seven years being the youngest record, G. A. Taylor pers. comm.), low reproductive rate (only one egg is laid per year), social monogamy, long-term pair bonds, biparental care of offspring and a long lifespan (perhaps 30-40 years, Aikman et al. 2001).

However, the Tāiko is difficult to observe and study because it is rare, pelagic and only comes to land during the breeding season where it is nocturnal and inhabits underground burrows in a remote location (Aikman et al. 2001; Johnston et al. 2003). Therefore, the mating system, mating behaviour and the relationships between individuals is difficult to determine by observation and biparentally inherited nuclear genetic markers provide extremely useful tools for this purpose. Genetic studies are most informative when they can be used in conjunction with biological and ecological data (Abbott et al. 2006). Monitoring of Tāiko activity and identity at burrows is performed every breeding season by direct observation, inspecting the inside of burrows with a small camera, determining burrow occupancy with 'knock-down fences' (i.e. sticks placed at the burrow entrance) and by infra-red video recording. Nuclear genetic data together with these observational data can provide knowledge of the

relationships between Tāiko, which can then assist conservation management (Aikman et al. 2001).

Knowledge of relatedness between individuals is important in understanding mating systems and behaviour. Not all petrels maintain their pair bond for life, but petrels generally show fidelity to their nest site and mate (Warham 1990). Tāiko are thought to be socially monogamous but extra-pair mating would be almost impossible to witness (Griffith et al. 2002). Nuclear DNA analyses of offspring and putative parents is the only reliable method to detect extra-pair mating providing it results in fertilisation (Avise 1996). Analysis using biparentally inherited nuclear DNA markers has detected extra-pair fertilisation in socially monogamous petrels, for example the Short-tailed Shearwater *Puffinus tenuirostris*, Antarctic Petrel *Thalassoica antarctica* and a number of albatross species (Austin and Parkin 1996; Lorentsen et al. 2000; Abbott et al. 2006; Burg and Croxall 2006). Conversely, four other studies of Procellariiformes have not detected extra-pair paternity (reviewed in Burg and Croxall 2006). For petrels, establishing a new pair bond and successfully breeding with a new partner can take a number of years (Warham 1990). Genetic parentage analysis can elucidate these aspects of the Tāiko mating system further.

Behaviours such as philopatry and kin recognition can also be examined using genetics in combination with observational data. Identifying the extent of philopatry has direct relevance for one of the Tāiko conservation management initiatives. Tāiko chicks are to be translocated to a secure breeding site within a predator-exclusion fence. It is anticipated that they will return to this area to breed. Knowledge of the fine-scale genetic structuring can increase understanding of the extent of natal philopatry (e.g. Temple et al. 2006). This could also aid conservation by suggesting a geographic distance from breeding burrows within which returning offspring are likely to be located.

Inbreeding depression can reduce the fitness and survival of individuals. This phenomenon can occur when closely related individuals breed, because it increases the probability that detrimental recessive traits will be homozygous in the progeny (Amos and Balmford 2001). Some Tāiko pairs have had a number of unsuccessful breeding attempts and one chick was born with a neck abnormality (G. A. Taylor pers. comm.). Identifying the relationships of the individuals involved could determine if inbreeding depression might contribute to such abnormalities.

An area where genetic research can perhaps be the most useful to conservation is in identifying the likelihood of undiscovered Tāiko burrows and in aiding their discovery (Aikman et al. 2001). It is essential for the continued survival of the species that active burrows are found so Tāiko can be protected from predators. Predation by introduced exotic species is a

serious threat to Tāiko and in some years all chicks have been lost to predators (Taylor 2000). Identifying parentage of prospecting non-breeding birds could suggest whether undetected pairs are breeding, i.e. if the prospectors are not offspring of known pairs. Also, identifying parentage and relationships of Tāiko caught from flight outside of the breeding area (and not associated with a geographic location) could also identify the existence of undetected breeding pairs and prioritise individuals for radio-telemetry tracking. In addition, search areas can be suggested by determining the location of related birds. This aspect also relates to understanding of philopatry.

In order to address questions regarding the mating system and specific relationships in Tāiko eight microsatellite DNA loci were genotyped for almost the entire known Tāiko population. Microsatellite DNA markers are well suited for this purpose because the mutation rates of these markers are several orders of magnitude greater than other DNA sequences (Wan et al. 2004). Parentage and sibship analyses were conducted to determine specific relationships between individual Tāiko. Fine-scale genetic structure was examined in the population by spatial autocorrelation analysis, to aid understanding of philopatric behaviour. In addition, pairwise relatedness within different groups was examined. Identifying Tāiko relationships contributes to knowledge of the mating system and behaviour and aids the search for undiscovered individuals. This has important relevance to the conservation of this critically endangered species.

Methods

Sample Collection and DNA Extraction

Blood samples were collected from almost all known Tāiko (caught since October 1996, N = 145). High molecular weight DNA was extracted by proteinase K digestion and a modified version of the phenol/chloroform method (Sambrook et al. 1989). Breast feather samples were taken from one Tāiko; genomic DNA was extracted from the base of the feather using a method adapted from Walsh et al. (1991).

Microsatellite DNA Marker Isolation and Testing

A genomic DNA library enriched for GT/CA, GA/CT, AAT/TTA, AAAG/TTTC and GATA/CTAT repeats was constructed using a protocol modified from Armour et al. (1994; Berry et al. 2003). In total, 2016 clones were screened for inserts containing microsatellites with ^{32}P-labelled dinucleotide, trinucleotide and tetranucleotide repeats. Positive clones (178) were

sequenced; 99 contained microsatellite DNA. Only nine had sufficient flanking sequence from which primers could be designed using the program Primer3 (Rozen and Skaletsky 2000). Four loci could be amplified consistently by polymerase chain reaction (PCR); three of these loci were polymorphic. The three polymorphic loci were genotyped for the whole population. However, one locus had null alleles and the polymorphism detected in another locus derived from a rare insertion and deletion. These two loci were subsequently excluded from further analysis. The locus retained for further analysis was Tch6, with the repeat motif $(GT)_8GC(GT)_6$ and PCR primers: Tch6F 5'-GTTTCTTGGTGGTGGCTGAAGGTGTATG-3' and Tch6R 5'-GCCATTTGAGAATGTTTCAGC-3'.

I also designed primers for nine microsatellite DNA loci isolated in Tāiko using a method modified from the one above (Hamilton et al. 1999; E. S. MacAvoy pers. comm.; J. Pringle unpubl. data). Of these nine loci, six could be amplified consistently but only one was polymorphic. The polymorphic locus used for further analysis was Tch25, repeat motif $(GT)_9$ and primers: Tch25F 5'-GTTTCTTTGGCCAAATCTGTGACCTTA-3' and Tch25R 5'-TGCAGTCAGTGTCAGGAAGC-3'.

Twenty-two microsatellite DNA loci isolated in other avian species were tested for amplification and polymorphism in Tāiko. To test for polymorphism, polymerase chain reactions included 2μM of fluorescein-12-dUTP (Roche) and 100μM of each dNTP (Bioline). PCR products could then be genotyped without the expense of fluorescently labelling the primers for initial polymorphism tests. Of the 22 loci tested, 14 loci could be amplified consistently and six were polymorphic. The polymorphic loci were Paequ3, Paequ8, Paequ13 (Techow and O'Ryan 2004), RBG18 and RBG29 (Given et al. 2002) and De33 (which is sex-linked in albatrosses, Burg 1999; Abbott et al. 2006).

Microsatellite DNA Loci Genotyping and Analysis

The Tāiko population was genotyped for eight microsatellite DNA loci that were polymorphic and could be used in further analysis. Forward primers included a 'PIG-tail' GTTTCTT sequence at the 5' end to encourage adenylation thereby reducing stutter peaks (Brownstein et al. 1996); all reverse primers were fluorescently labelled. Reactions totalled 10μl and included 1X PCR Buffer (Invitrogen), 1.5mM $MgCL_2$ (Invitrogen), 200μM of each dNTP (Bioline), 0.2μM of each primer (Sigma or Applied Biosystems), 0.5 U of Platinum® Taq DNA polymerase (Invitrogen) and 50-200ng of DNA. A negative control (with no added DNA) was included in each set of PCRs. The following PCR profile was performed in a BIO-RAD iCycler, with slight variations for different loci: 94°C for 3 mins, then 30 cycles of 94°C for 30 sec, 55°C-57°C for 45 sec, 72°C for 45 sec and a final extension at 72°C for 5 min. The annealing

temperature was 50°C for Paequ8, 57°C for Tch6 and 55°C for all other loci. PCR products were run on a 3730 DNA analyser (Applied Biosystems) by the Allan Wilson Centre Genome Service.

Electrophoretic profiles were viewed using GeneMapper® 3.7 software (Applied Biosystems) and genotypes were scored manually. When peaks were ill defined, the PCR was repeated and the product run at a higher concentration. Genotyping results from two poor-quality samples were deemed unreliable and excluded from further analysis. The presence of genotyping errors caused by null alleles, large-allele dropout and/or scoring of stutter peaks were tested for by examining deviation from Hardy-Weinberg equilibrium using the program Micro-checker 2.2.3 (van Oosterhout et al. 2004).

Observed and expected heterozygosities were calculated for each autosomal microsatellite DNA locus using Arlequin 3.1 (Excoffier et al. 2005). Deviations from Hardy-Weinberg equilibrium were calculated using exact tests based on contingency tables (Guo and Thompson 1992) and deviations from linkage equilibrium were calculated with likelihood ratio tests using Arlequin 3.1 (Slatkin and Excoffier 1996; Excoffier et al. 2005). Sequential Bonferroni corrections for multiple tests were used to adjust *P* values for possible type I errors (Rice 1989). The probability of identity and probabilities of excluding unrelated parents using all autosomal loci was estimated using Cervus 3.0.3 (Kalinowski et al. 2007).

Analysis of Pairwise Relatedness

Mean pairwise relatedness estimates were obtained for groups including paired/breeding birds, unpaired/non-breeding birds and Tāiko caught in flight but not on land (using GenAlEx 6, Lynch and Ritland 1999; Peakall and Smouse 2006). The means were compared with 999 permutations and 999 bootstraps. Analyses were performed both including and excluding the locus showing linkage disequilibrium (i.e. Paequ3).

Parentage Analysis

Parentage analysis was performed with likelihood equations using Cervus 3.0.3 (which can tolerate weak linkage between loci, Kalinowski et al. 2007). Analysis considered the number of candidate parents, the proportion of candidates sampled, allele frequencies, error rate and completeness of genetic data, with genotypes of the offspring and potential parents. A log-likelihood ratio (LOD score) was calculated for the likelihood that candidate parents were true parents. The two most likely parent pairs were evaluated by comparison of the difference in their LOD scores with a criterion (Δ LOD) established by simulation. If the difference in LOD scores of the parent pairs was larger than the criterion, the assigned parentage was correct

with 95% strict confidence or 80% relaxed confidence (Marshall et al. 1998; Kalinowski et al. 2007). When both parents could not be determined the same likelihood method was used for maternity or paternity alone. A simulation was run with the following parameters: 10 000 cycles, 90% of candidate parents sampled, 99.8% of loci typed and a genotyping error rate of 1%.

Sibship Analysis

Analysis of possible sibling relationships was performed by calculating the likelihood for a primary hypothesis of full sibship and a null hypothesis of no relationship between two individuals, using Kinship 1.3.1 (Goodnight and Queller 1999). Variables *(r)* defined the probabilities that individuals in a pair share an allele by direct descent from their parents. For the hypothesis of full sibship, variables are 0.5 for each of the mother and father. For the null hypothesis of no relationship, variables are defined as 0 for each parent. Given the hypotheses, the likelihood of the genotype combination observed in two individuals being the result of the relationship specified is calculated using the *r* values and population frequencies. Allele frequencies were corrected for bias by specifying groups of chicks known to be full siblings. Frequencies were then calculated excluding those chicks thought to be close relatives of the individuals for which sibship analysis was performed. The significance level of the likelihood ratio between primary and null hypotheses was calculated empirically by simulation (1 000 000 cycles, Goodnight and Queller 1999). Sibship analyses were under the assumptions of no linkage disequilibrium, no inbreeding and no mutation (Goodnight and Queller 1999). Other hypotheses tested were half-sibship versus no relationship, full-sibship versus half-sibship and half versus full-sibship. Similar analyses were also performed using hypotheses of full versus half sibship given a candidate mother for some individuals.

Spatial Autocorrelation Analysis

Spatial autocorrelation analysis was used to test for fine-scale genetic structuring in microsatellite DNA alleles in Tāiko using the software GenAlEx 6 (Peakall and Smouse 2006). The procedure was as in chapter four for both 'global' and 'local' analyses. Global analyses grouped pairwise genetic comparisons into geographic distance classes and included Tāiko adults that had been associated with a specific geographic location (n = 63). Local analyses were used to detect clusters of genetic similarity using subsets of the data (Double et al. 2005). Sexes were analysed both separately and together for both global and local analyses.

Results

Microsatellite DNA Loci

One microsatellite DNA locus genotyped was found to be sex-linked (De33, Abbott et al. 2006). This locus was therefore excluded from analyses, but used manually for exclusion in parentage analysis. The seven autosomal loci genotyped had two to nine alleles (Table 6.1). Linkage involving locus Paequ3 was observed (i.e. Paequ3 and Paequ13, Paequ3 and RBG29). Rejection of linkage disequilibrium can be due to departure from Hardy-Weinberg equilibrium (HWE) (Excoffier and Slatkin 1998). Locus Paequ3 was not in agreement with HWE without Bonferroni correction (P = 0.036). All other loci were in HWE both with and without correction. Also, there was no deviation from HWE at any locus using a goodness-of-fit test (using Cervus 3.0.3, Kalinowski et al. 2007). No genotyping errors caused by null alleles, large allelic dropout and/or scoring of stutter peaks were detected (using Microchecker 2.2.3, van Oosterhout et al. 2004).

Table 6.1 Number of alleles *(A)*, observed *(H_O)* and expected *(H_E)* heterozygosities for Tāiko individuals *(N)* for seven microsatellite DNA loci. Hardy-Weinberg equilibrium *P* values for each locus are shown *(P),* as are probabilities of exclusion *(PE)* of unrelated parents combined across all seven autosomal microsatellite DNA loci (calculated using Cervus 3.0).

Locus	*N*	*A*	*H_O*	*H_E*	*P*	*PE* First parent	*PE* Second parent*	*PE* Parent pair
Tch6	143	2	0.049	0.048	1.000	0.001	0.023	0.045
Tch25	141	4	0.482	0.527	0.370	0.139	0.249	0.379
Paequ3	143	9	0.881	0.850	0.036	0.529	0.695	0.864
Paequ8	143	2	0.077	0.074	1.000	0.003	0.036	0.067
Paequ13	142	3	0.472	0.442	0.398	0.097	0.232	0.373
RBG18	143	2	0.168	0.154	0.599	0.012	0.071	0.127
RBG29	143	5	0.636	0.693	0.150	0.264	0.428	0.600
Mean	143	3.857	0.395	0.398				
Combined						**0.7343**	**0.9120**	**0.9835**

*when genotype of first parent is known

Parentage and Sibship

Sibship analysis was conducted both including and excluding the locus showing linkage disequilibrium (i.e. Paequ3). Results were very similar in both instances therefore very similar conclusions could be made. Sibship results presented are those excluding Paequ3.

Exclusion probabilities were adequate for parentage analysis of Tāiko (Table 6.1) and the probability of genotype identity of two randomly selected individuals was low (0.0006, calculated using Cervus 3.0.3, Kalinowski et al. 2007). Parentage analysis resulted in a much higher exclusion probability when one parent was known (*PE* = 0.912, Table 6.1). Females observed in burrows during the breeding season of a chick's birth were assumed to be mothers and genotypes were consistent with this. Suspected mothers observed in the same burrow (but during a different season) were checked by likelihood analysis. In almost all instances the putative mother was the most likely mother (or only option) when mitochondrial DNA haplotype was also considered. For other chicks, mitochondrial and microsatellite genetic data were consistent with the putative mother (from field observations) as the real mother (if not the most likely). Therefore there was no evidence of nest parasitism or mutation. Once these mothers were identified, they could then be used to increase the power of paternity analysis.

In only one case was a genetic mismatch detected in 55 genotype comparisons between putative parents (identified by field observation) and chicks. One mismatch between daughter and putative father was discovered. Four potential fathers with no mismatch were identified, however these potential fathers occupied burrows far from the burrow in question. It is possible that the true father was not sampled, or that a mutation or genotyping error occurred. Genotyping of the chick and father was repeated – with the same result. The homozygous alleles present in the putative father and chick differ by 4bp, so large-allelic dropout is unlikely. The male was not identified in the burrow that season but was present in previous and subsequent seasons. He has produced offspring with the mother in previous breeding seasons.

Three of the unpaired non-breeding Tāiko (not banded as chicks, n =14) could be offspring of known breeding pairs. The blood sample for the potential father of three other non-breeders was unable to be genotyped. Eight of the non-breeders are not offspring of known pairs (past or present). In total, potential mothers of seven unpaired non-breeders were identified. Of these seven potential maternal relationships, three seem likely due to close geographic proximity of potential mother and offspring. However, seven unpaired non-breeders have no possible mothers (without genetic mismatch) and are not full siblings of each other (one dyad could be half-sibs, $P < 0.05$).

There have been 21 Tāiko caught in flight and not found on land. Of these birds, only 4 could be the offspring of known Tāiko breeding pairs. Seven birds caught in flight could be offspring of known females, but not known breeding pairs (past or present). Fathers could be males that were undetected before research began. Ten of the birds caught only from flight (and not on land) could not possibly be offspring of any known female without genetic mismatch.

Pairwise Relatedness

Mean pairwise relatedness within the unpaired non-breeding group was not significantly different from the entire population mean. However, Tāiko caught in flight and not on land were significantly more closely related to each other than was the rest of the population (P = 0.040 including Paequ3, P = 0.044 excluding Paequ3).

Spatial Autocorrelation

A global analysis detected significant spatial structure among the microsatellite DNA genotypes of Tāiko i.e. when comparisons were made across the entire adult population for which geographic locations were known (n = 63). Global spatial autocorrelation analysis resulted in a significant positive autocorrelation coefficient r-value for the 50m distance class (P = 0.001; Fig. 6.1). The same result was obtained when the locus showing linkage disequilibrium (i.e. Paequ3) was excluded from analysis (P = 0.05). Significant global spatial structure was not detected when the sexes were analysed separately.

Global spatial autocorrelation analysis does not describe the relationships between different geographical groups or individuals. Clusters of genetic autocorrelation can exist even when there is little or no significant global spatial structure (Double et al. 2005). Therefore, a local spatial autocorrelation analysis was implemented. This analysis requires specification of the number of individuals to be regarded as 'nearest-neighbours' to compare to a pivotal individual. Nearest neighbour distance calculations suggested that four nearest neighbours was the most appropriate parameter since the mean distance is 61m (when three outliers are excluded, chapter four, see Fig. 4.4), which typically included birds within the same burrow group (Table 6.2). Burrow groups are units that have been arbitrarily defined based on geographic proximity. Local correlation values *(lr*, for four nearest neighbours, 4NN) were significantly positive ($P < 0.05$) for 8% of the 63 adult Tāiko (of both sexes) in a one-tailed test using conditional permutations. Standard and conditional permutations produced similar results over the range of 1 to 10 nearest neighbours, for analysis including all adults. The number of significant *lr* values ranged from 5 to 12 for both types of permutation (for 2 to 10

nearest neighbours). The pattern was therefore consistent over the 2 to 10 nearest neighbour classes. Exclusion of the locus showing linkage disequilibrium (i.e. Paequ3) actually increased the number of significant *lr* values in the analysis including all adults. Therefore, the more conservative estimates obtained by including this locus in analysis are reported (Table 6.2).

For 40 adult Tāiko males, 13% had significant local correlation values *(lr)* in a one-tailed test using conditional permutations ($P < 0.05$, for 4NN). One to eight *lr* values were significant over the range of two to ten nearest neighbours (Table 6.2). In contrast, for the 23 adult Tāiko females, very few had significant ($P < 0.05$) *lr* values – either one or zero (using a one-tailed test with conditional permutations, Table 6.2). Although the data set is small ($n = 23$), results suggested very little or no spatial genetic structure for females.

Table 6.2 Results of a two-dimensional local spatial autocorrelation analysis in Tāiko (using a one-tailed test with conditional permutations in GenAlEx 6).

Number of nearest neighbours	1	2	3	4 [a]	5	6	7	8	9	10
Mean geographic distance (m) [b]	11	16	38	61	80	96	133	267	310	322
Number of individuals with significant [c] *lr* [d] values										
All adults ($n = 63$)	3	5	7	5	6	7	8	11	12	12
Males ($n = 40$)	4	1	3	5	5	6	8	2	3	5
Females ($n = 23$)	3	1	0	1	1	0	1	0	0	-

[a] *Shaded column highlights values determined using four nearest neighbours*

[b] *For calculation of mean geographic distance (m = metres) three outliers in northern and southern areas were excluded (see Fig. 4.4)*

[c] *Significant at $P < 0.05$*

[d] *lr = local correlation values*

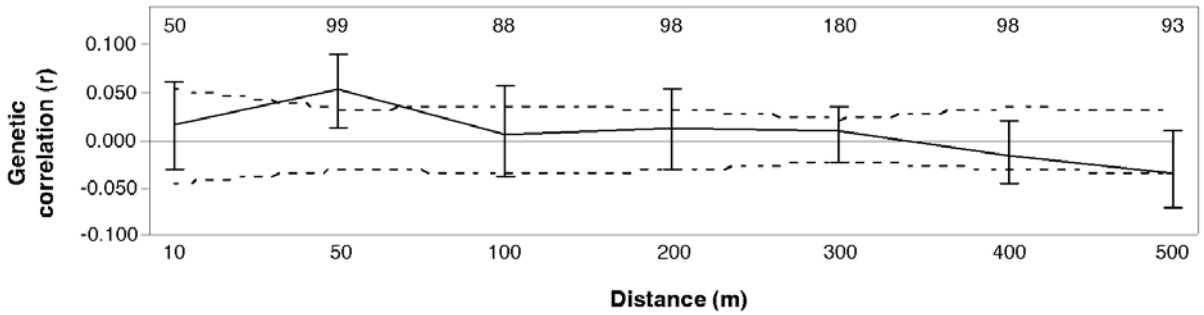

Figure 6.1 Correlogram plot of the genetic correlation coefficient *(r)* as a function of distance (m) for adult Tāiko associated with known locations (*n* = 63). Dashed lines represent the permuted 95% confidence intervals; bars represent bootstrapped 95% confidence error. Numbers above data points indicate number of pairwise comparisons within each distance class.

Discussion

Variation

Variation in microsatellite DNA markers was reasonably low in Tāiko. Fourteen loci were monomorphic and the seven polymorphic autosomal loci genotyped had two to nine alleles per locus. Limited genetic variability measured by microsatellite DNA markers has been found in albatross species compared with non-Procellariiform species (Bried et al. 2007). However it is problematic to determine microsatellite DNA variation levels relative to other species because different loci are compared. Studies tend to use the most polymorphic loci available and this is related to availability and effort in marker isolation. Nevertheless, the level of variation in the seven autosomal loci was sufficient to determine parentage and sibling relationships in Tāiko.

Mating systems

Species with life history traits such as is typical for Procellariiformes (e.g. long lifespan, long-term pair bonds and biparental care) are generally expected to be both genetically and socially monogamous (Abbott et al. 2006). This appears to largely be the case for Tāiko. There are well-established Tāiko pairs that have raised chicks almost every year (since their burrow was discovered) without any extra-pair paternity (i.e. six, seven and eight chicks fledged). Maternity was consistent with social monogamy so no incidences of nest parasitism were detected. Extra-pair paternity was detected at a very low rate of 0.02 (*n* = 55). This rate is much lower than that found in some other Procellariiform species such as albatross (Abbott et al. 2006). The rate of extra-pair paternity (EPP) may not be directly comparable to that

found in other studies since in this study more than one chick from each pair was included. Nevertheless, it can be concluded that EPP in Tāiko is rare.

It can take petrels pairs a number of years to breed after the pair bond is established. Two cases were detected where a female skipped only a single breeding season between producing chicks with different fathers. For males who lost a partner 2 and 3 breeding seasons were missed (in two different cases) before successful breeding again. This could have been due to time taken to attract another mate rather than time to establish pair bond for breeding. These results suggest that once a mate is lost, breeding with another mate can happen relatively quickly with only one season missed.

Two possible instances of inbreeding depression were detected by identification of genetic relationships. A female mated with her sibling and had an egg failure at first breeding attempt. In the second attempt they produced a chick with physical abnormality (a neck weakness/deformity). There was no evidence of infection, inflammation or parasites (K. McInnes pers. comm.). The neck abnormality was corrected with a brace and the chick fledged successfully. This could be anecdotal evidence of inbreeding depression however chicks produced by this pair should be carefully monitored. Another potential instance of inbreeding depression involved a likely mother-son Tāiko pair that had egg failures for four years since their burrow was discovered, with no chicks fledged. The female has since paired with another male and is breeding successfully.

Behaviour

One advantage of the small Tāiko population is that behaviour and movements of individuals can be studied at a detailed level and precise biological and ecological data can be collected. Surprisingly, two Tāiko females were found together in a burrow. This has not been observed in the closely related well-studied Grey-faced Petrel *(Pterodroma macroptera gouldi)* because females do not tolerate other females and will aggressively defend their burrow (G. A. Taylor pers. comm.). The two Tāiko females were revealed as mother and daughter by microsatellite parentage analysis supported by the fact they share a mitochondrial haplotype only found in birds in this family. It is interesting that that the younger female was tolerated – perhaps suggesting offspring recognition. Odour-based recognition has been detected in another Procellariiform species, the Antarctic Prion *(Pachiptila desolata*, Bonadonna and Nevitt 2004).

Fine-scale genetic structuring indicates Tāiko are philopatric within 50m, with males being more philopatric than females. Tāiko conservation plans include searching around known active burrows for other inhabited burrows. Taylor (2000) recommended searches within 100m of burrows whereas Aikman et al. (2001) suggested 20m. Genetic results suggest 50m

around each burrow is a more appropriate distance to search, since philopatric offspring produced at known burrows could be found within this range.

Other Undiscovered Birds

There is an excess of non-breeding males in the Tāiko population (chapter five). It was hypothesised that since Tāiko are philopatric, the non-breeding birds could be offspring of established Tāiko pairs in close geographical proximity to where the non-breeders are found. However, results show that many of the non-breeding Tāiko are not offspring of known Tāiko breeding pairs (past or present). There are a number of possible explanations for this result. There could have been mate switching or extra-pair paternity in the case of the parents of the non-breeders for whom a potential mother was identified. This seems unlikely since Tāiko form long-term bonds and are socially monogamous (G. A. Taylor unpubl. data). This study detected one possible incidence of extra-pair paternity, suggesting it is rare. A more likely explanation is that the fathers of these non-breeders died before sampling began, or before the burrow was discovered.

Of the non-breeding Tāiko, seven have no possible mothers yet detected in the population. There are two likely explanations. Firstly, the mothers of these non-breeders could have died before sampling began, or before the burrow was discovered. This seems likely for one bird because statistically significant full siblings have been identified in reasonably close geographic proximity. If correct, this would suggest the birds are old, which is of concern because these birds could be unable to reproduce due to difficulties in finding mates rather than being young pre-breeding birds. Secondly, the parents of the non-breeders could be breeding elsewhere in an undiscovered location. Mean pairwise relatedness estimates suggest that the non-breeders and their parents are part of the known Tāiko colony rather than forming a separate group.

More evidence to support the likely existence of further undiscovered breeding pairs is found in examining possible parentage of Tāiko caught in flight (and not on land). In addition to seven Tāiko caught with mitochondrial haplotypes only found in birds not associated with a geographic location (chapter four), there are three others for whom microsatellite data excludes all known females as potential mothers. In addition, mean pairwise relatedness estimates indicated the Tāiko caught in flight were more closely related to each other than was the known Tāiko population and could exist in a separate breeding colony or group. This result emphasises the importance of continuing to catch and track Tāiko in flight because they could lead to another breeding colony.

In total, of the unpaired non-breeding birds and birds that have not been found on land, 17 Tāiko were identified that are not offspring of any known female. Some individuals have statistically significant full siblings so the minimum number of potential undiscovered females is likely to be 14. This result is of great importance for conservation as it is likely more Tāiko are breeding in unknown areas.

The microsatellite data could aid the tracking of Tāiko. Paternity analysis suggests four of the birds caught in flight are offspring of a known pair. Since Tāiko tend to be philopatric these birds are likely to return to an area near their natal burrow. Seven Tāiko could be offspring of known females, they too may be found near their potential natal burrow. By identifying potential natal areas, microsatellite DNA data can aid tracking efforts by suggesting areas to search. In addition, the identification of candidate mothers of unpaired non-breeders suggests burrows where searches could be undertaken around.

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References

Abbott CL, Double MC, Gales R, Cockburn A (2006) Copulation behaviour and paternity in Shy Albatrosses *(Thalassarche cauta)*. Journal of Zoology 270:628-635

Aikman H, Davis A, Miskelly C, O'Conner S, Taylor GA (2001) Chatham Island Tāiko Recovery Plan 2001-2011. Threatened Species Recovery Plan 36. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/TSRP36.pdf

Amos W, Balmford A (2001) When does conservation genetics matter? Heredity 87:257-265

Armour JAL, Neumann R, Gorbert S, Jeffreys AJ (1994) Isolation of human simple repeat loci by hybridisation selection. Human Molecular Genetics 3:599-605

Austin JJ, Parkin DT (1996) Low frequency of extra-pair paternity in two colonies of the socially monogamous Short-tailed Shearwater *Puffinus tenuirostris*. Molecular Ecology 5:145-150

Avise JC (1996) Three fundamental contributions of molecular genetics to avian ecology and evolution. Ibis 138:16-25

Berry O, Gleeson DM, Sarre SD (2003) Microsatellite DNA markers for New Zealand skinks. Conservation Genetics 4:411-414

Bonadonna F, Nevitt GA (2004) Partner-specific odor recognition in an Antarctic seabird. Science 306:835

Bried J, Nicolaus M, Jarne P, Dubois M-P, Jouventin P (2007) Population biology of the Wandering Albatross *(Diomedea exulans)* in the Crozet and Kerguelen archipelagos, southern Indian Ocean, approached through genetic and demographic methods. Journal of Zoology 272:20-29

Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. BioTechniques 20:1004-1010

Burg TM (1999) Isolation and characterization of microsatellites in albatrosses. Molecular Ecology 8:335-346

Burg TM, Croxall JP (2006) Extrapair paternities in Black-browed *Thalassarche melanophris*, Grey-headed *T. chrysostoma* and Wandering Albatross *Diomedea exulans* at South Georgia. Journal of Avian Biology 37:331-338

Crockett DE (1994) Rediscovery of Chatham Island Tāiko *Pterodroma magentae*. Notornis (Supplement) 41:49-60

Double M, Peakall R, Beck N, Cockburn A (2005) Dispersal, philopatry, and infidelity: dissecting local genetic structure in Superb Fairy-Wrens *(Malurus cyaneus)*. Evolution 59:625-635

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50

Excoffier L, Slatkin M (1998) Incorporating genotypes of relatives into a test of linkage disequilibrium. American Journal of Human Genetics 62:171-180

Given AD, Mills JA, Baker AJ (2002) Isolation of polymorphic microsatellite loci from the Red-billed Gull *(Larus novaehollandiae scopulinus)* and amplification in related species. Molecular Ecology 2:416-418

Goodnight KF, Queller DC (1999) Computer software for performing likelihood tests of pedigree relationship using genetic markers. Molecular Ecology 8:1231-1234

Gowaty PA, Gibbs HL (1993) DNA fingerprinting in avian behavioural ecology: two cultures arise. Auk 110:152-155

Griffith SC, Owens IPF, Thuman KA (2002) Extra pair paternity in birds: a review of interspecific variation and adaptive function. Molecular Ecology 11:2195-2212

Guo S, Thompson E (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372

Hamilton MB, Pincus EL, Di Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. BioTechniques 27:501-507

Hitchmough R, Bull L, Cromarty P (comps) (2007) New Zealand Threat Classification System Lists 2005. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/sap236.pdf

Imber MJ, Taylor GA, Tennyson AJD, Aikman HA, Scofield RP, Ballantyne J, Crockett DE (2005) Non-breeding behaviour of Magenta Petrels *Pterodroma magentae* at Chatham Island, New Zealand. Ibis 147:758-763

IUCN (World Conservation Union) (2006) IUCN Red List of Threatened Species 2006. IUCN, Gland, Switzerland. Available from http://www.iucnredlist.org (accessed June 2007)

Johnston RB, Bettany SM, Ogle RM, Aikman HA, Taylor GA, Imber MJ (2003) Breeding and fledging behaviour of the Chatham Tāiko (Magenta Petrel) *Pterodroma magentae* and predator activity at burrows. Marine Ornithology 31:193-197

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099-1106

Lorentsen S-H, Amundsen T, Anthonisen K, Lifjeld J T (2000) Molecular evidence for extrapair paternity and female-female pairs in Antarctic Petrels. Auk 117:1042-1047

Lynch M, Ritland K (1999) Estimation of pairwise relatedness with molecular markers. Genetics 152:1753-1766

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Molecular Ecology 7:639-655

Peakall R, Smouse P (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223-225

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Bioinformatics Methods and Protocols: Methods in Molecular Biology (eds. Krawetz S, Misener S), pp. 365-386. Humana Press, New Jersey

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York

Schreiber EA, Burger J (2002) Biology of Marine Birds. CRC Press, Boca Raton

Scofield RP (Aug 2004) Population assessment of the endangered Chatham Island Tāiko. In: Third International Albatross and Petrel Conference, Montevideo, Uruguay

Slatkin M, Excoffier L (1996) Testing for linkage disequilibrium in genotypic data using the EM algorithm. Heredity 76:377-383

Taylor GA (2000) Action Plan for Seabird Conservation in New Zealand Part A: Threatened Seabirds. Threatened Species Occasional Publication 16. Department of Conservation, Wellington http://www.doc.govt.nz/upload/documents/science-and-technical/TSOP16.pdf

Techow NMSM, O'Ryan C (2004) Characterization of microsatellite loci in White-chinned Petrel *(Procellaria aequinoctialis)* and cross-amplification in six other Procellariiform species. Molecular Ecology Notes 4:33-35

Temple HJ, Hoffman JI, Amos W (2006) Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the White-breasted Thrasher, *Ramphocinclus brachyurus*. Molecular Ecology 15:3449-3458

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535-538

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 10:506-513

Wan Q-H, Wu H, Fujihara T, Fang S-G (2004) Which genetic marker for which conservation genetics issue? Electrophoresis 25:2165-2176

Warham J (1990) The Petrels Their Ecology and Breeding Systems. Academic Press, London

Wayne RK, Morin PA (2004) Conservation genetics in the new molecular age. Frontiers in Ecology and the Environment 2:89-97

Chapter Seven

The Magenta Petrel - Solving a 140 Year Old Mystery

Abstract

A lone petrel was shot from the decks of an Italian warship (the *'Magenta'*) while it was sailing the South Pacific Ocean in 1867, far from land. The species, unknown to science, was named the 'Magenta Petrel' *(Pterodroma magentae)*. No other specimens of this bird were ever collected and the species it represented remained a complete enigma for over 100 years. DNA sequence of the mitochondrial cytochrome *b* gene from samples of the Magenta Petrel were included in phylogenetic analyses with sequences from all species in the *Pterodroma* subgenus and a number of other morphologically similar petrels. Phylogenetic analyses (Bayesian, maximum likelihood and maximum parsimony) grouped the Magenta Petrel and Chatham Island Tāiko in a monophyletic clade with strong posterior probability and bootstrap support. These results indicate the Tāiko and the Magenta Petrel are a single species. Furthermore, given the collection location of the Magenta Petrel, our results suggest that the Chatham Island Tāiko forages far into the Pacific Ocean (near South America) and/or that as yet to be discovered populations of Tāiko exist near South America. Both of these possibilities have significant conservation implications for the Tāiko, one of the world's rarest seabirds.

Introduction

On the 22nd July 1867 a solitary petrel was seen flying fast near an Italian warship (Godman 1910). The ship was the H. I. M. S. '*Magenta*', captained by Victor Arminjon. The naturalist aboard the ship, Dr Henry Hillyer Giglioli, shot the lone petrel and realised it was of a species unknown to Western science. At the time the specimen was collected the ship was located in the South Pacific Ocean south of the Tubai Islands (39°38'S, 125°58'W, Giglioli and Salvadori 1869; Fig. 7.1). Giglioli saw the same species weeks later when positioned southeast of Easter Island/Rapa Nui (3rd August 1867, 32°23'S, 92°39'W) and again north of the Juan Fernandez Islands near Chile (31st August 1867, 26°07'S, 88°50'W), but described it as 'rare' (Giglioli and Salvadori 1869; Fig. 7.1).

The lone bird was named the 'Magenta Petrel' after the ship, the first Italian warship to circumnavigate the world. The bird was described as a new species *(Aestrelata magentae,* Giglioli and Salvadori 1869; *Oestrelata magentae,* Salvin 1876). On the same voyage, type specimens of three other congeneric species were obtained (Giglioli and Salvadori 1869). Since that time further specimens of these three species have been collected and their breeding sites located but the Magenta Petrel remained an enigma, never being recorded or collected again.

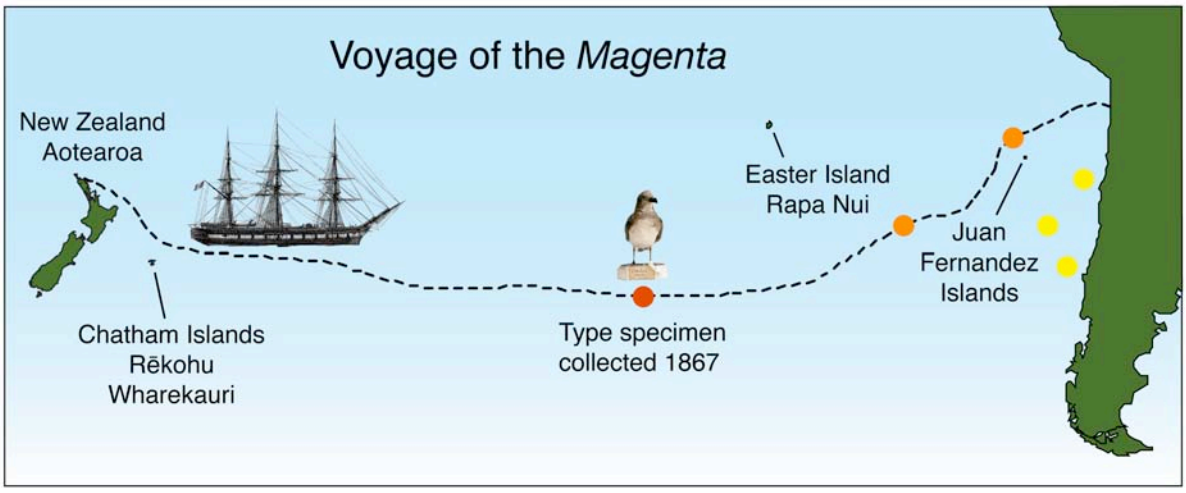

Figure 7.1 Voyage of the *Magenta* across the South Pacific Ocean in 1867 (represented by the dashed line). The approximate location of the collection site of the Magenta Petrel type specimen is indicated with a red circle. Sightings from the voyage are indicated with orange circles, yellow circles indicate the approximate locations of reported sightings of the Tāiko near South America. The picture of the ship "Regia Pirocorvetta Magenta" (Giglioli 1875) is reproduced with permission from the Alexander Turnbull Library, Wellington, New Zealand (from whom permission must be obtained before any re-use of this image).

The Magenta Petrel type specimen was initially housed in the Zoological Museum of the University of Torino in Italy (Imber et al. 1998; Fig. 7.2A). The bird was evidently a first-year juvenile as it has near perfect plumage without any hint of moult or worn feathers (M. J. Imber pers. comm.). A petrel expert Osbert Salvin commissioned the well-known bird artist John Gerrard Keulemans to produce two illustrations of the specimen (Salvin 1876; Godman 1910; Fig. 7.2B). The colouring of the bird in the plates (by Dr Sharpe's daughters, Godman 1910) is very accurate (Bourne 1964). However, not surprisingly the posture and location of the bird is unnatural and presumably reflects only the artist's imagination as the bird had only ever been seen in flight. In his 'Monograph of the Petrels', Frederick Du Cane Godman calls the Magenta Petrel 'Giglioli's Fulmar's (1910).

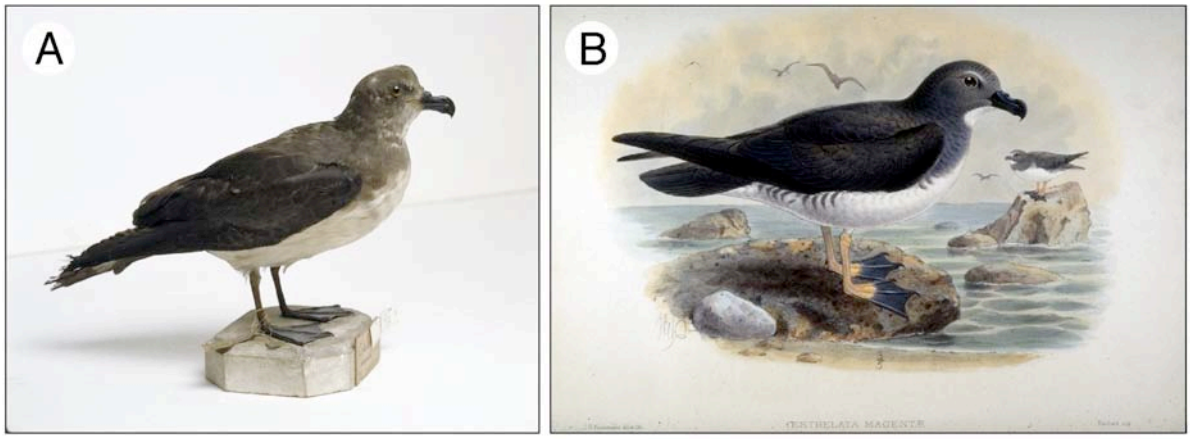

Figure 7.2 The Magenta Petrel type specimen **A** Photograph by K. Guldbrandsen
B Coloured plate by J. G. Keulemans (Godman 1910: 202)

A bird expert wrote that, "all petrels are puzzling in almost every way" (Newton in Godman 1910). This statement is valid generally and is certainly true with respect to the Magenta Petrel. The type specimen is morphologically very similar to a number of other petrels and its relationship to these species has been the subject of conjecture. Of the species described as similar to the Magenta Petrel, only the Tahiti Petrel *(Pseudobulweria rostrata)* belongs to a genus other than *Pterodroma.* Imber (1985) recognised 29 *Pterodroma* species, with the following species belonging to the *Pterodroma* subgenus: *magentae, ultima, solandri, macroptera, lessonii, mollis, madeira, feae, incerta, hasitata* and *cahow*. This *Pterodroma* subgenus is monophyletic in phylogenetic analyses conducted using the mitochondrial cytochrome *b* gene (Nunn and Stanley 1998). Supertrees show this concordance and strongly support this monophyletic group (Kennedy and Page 2002).

The Question of the Identity of the Magenta Petrel

In 1956, William Bourne found the Magenta Petrel type specimen in surprisingly good condition in the Turin museum attic, despite the attic having been damaged by bombing in the Second World War (Crockett 1994). He later examined subfossil petrel bones collected from the Chatham Islands. Bourne then proposed that the Magenta Petrel was the same species as one of the most commonly represented bones in the collection and that that species was the Chatham Island Tāiko (Bourne 1964). These suggestions were further supported by Dr Robert Falla who had written accounts of the Tāiko and Robert McClurg a Chatham Islander who had seen the Tāiko (Bourne 1964). However, no skins or specimens of the Chatham Island Tāiko were known to Western science for comparison and the Tāiko was thought to have gone extinct. Despite this common belief, William Bourne proposed that the Tāiko could possibly be still surviving in low numbers on the Chatham Islands (Bourne 1964). This suggestion was proven correct when, after a 34-year effort by David Crockett, the Tāiko was rediscovered. On the night of New Years day in 1978, he captured two Tāiko (Crockett 1994). Despite the rediscovery of the Tāiko, Fullagar and van Tets maintain that the Magenta Petrel is merely a form of the Phoenix Petrel *(P. alba*, Harrison 1983).

Phylogenetic analyses were conducted to determine the specific status of the Magenta Petrel. A partial cytochrome *b* sequence was obtained from the Magenta Petrel specimen using ancient DNA techniques. This Magenta Petrel sequence was compared with equivalent sequences of similar species deposited in GenBank®. Additional sequences of *Pterodroma alba, P. arminjoniana, P. ultima, P. cervicalis* and *P. macroptera gouldi* were obtained to include in the phylogenetic comparison. The phylogeny therefore included all species recorded as being visually similar to the Magenta Petrel, including the Tāiko and those species that are closely related to the Tāiko (in the same subgenus, determined by genetic and morphological methods).

Methods

DNA Samples and Extraction

Tissue samples were obtained from the Magenta Petrel type specimen that is stored at the Museo Regionale di Scienze Naturali Torino by scraping skin from the base of the tarsus under the foot. A feather sample was also obtained from this specimen. Feather samples were obtained from *Pterodroma alba* (Av3886)*, P. cervicalis* (Av1313)*, P. externa* (Av1305)*, P. ultima* (Av1320) and a specimen labelled *Pseudobulweria rostrata* (Av14948) housed at

the Canterbury Museum, Christchurch. In addition, blood samples of *Pterodroma macroptera gouldi* from Kauwahaia Island, Ihumoana Island and Whakatane were collected and stored in Queen's lysis buffer (Seutin et al. 1991)

DNA extraction of feather and tissue samples and polymerase chain reaction (PCR) set up was performed in a physically isolated dedicated ancient DNA laboratory. Appropriate measures were taken to prevent contamination (details in appendix C). Small pieces of tissue or the base of feathers were incubated, rotating overnight at 55°C in 200μl extraction buffer (100mM Tris-HCl pH8.0, 100mM NaCl, 1mM EDTA) with 8μl of 200mg/ml 1M dithiothreitol (DTT), 20μl 10% sodium dodecyl sulfate (SDS) and 20μl proteinase K (20mg/ml). Extraction negative controls were included at a minimum ratio of one negative per three samples. The next day, an equal volume of phenol:chloroform:isoamyl alcohol was added, mixed, then tubes centrifuged at 20800 rcf for 1min. DNA was extracted from the supernatant using a QIAamp® DNA Mini Kit (Qiagen), following the post-lysis tissue extraction protocol.

DNA was extracted from three blood samples of *Pterodroma macroptera gouldi* by proteinase K digestion and a modified version of the phenol/chloroform method (Sambrook et al. 1989).

DNA Amplification and Sequencing

Three regions of cytochrome *b*, corresponding to variable regions identified in the Chatham Island Tāiko population (chapter two), were amplified by PCR. Primers included L14863 (Nunn et al. 1996) and HCytB21-55; LCytB432-571 and HCytB432-571; and LCytB679-780 and HCytB679-780 (primer and reaction mixture details in chapter three). For the third region, the Magenta Petrel sample was amplified by PCR containing 50mM Tris-HCl (pH8.8) and 20mM $(NH_4)_2SO_4$ instead of Invitrogen buffer. Amplification was carried out as in chapter three, with annealing temperatures of 52°C and 50°C in the first and second set of cycles respectively. DNA extracted from blood samples was amplified using primers L14863 and HTāikoThr2 (details in appendix A). The PCR products were purified using a QIAquick® PCR purification kit (Qiagen) then sequenced on an Applied Biosystems 3730 DNA analyser by the Allan Wilson Centre Genome Service. For the ancient DNA, the first cytochrome *b* region PCR products were sequenced with primer L14863 twice, for the other two regions both light and heavy strand primers were used. Sequencing primers for *P. macroptera gouldi* included L14863, LCytB432-571, HCytB432-571 and HTāikoThr2 to obtain the entire cytochrome *b* sequence. Sequences were edited and aligned using Sequencher™ version 4.2.2 (GeneCodes). Sequences will be deposited in the GenBank® database.

Replication

DNA from a feather and a tissue sample from the Magenta Petrel was extracted, amplified and purified for sequencing at another dedicated ancient DNA laboratory by Judith H. Robins (University of Auckland) using the protocols described above.

Reference DNA Sequences Included in Analyses

Cytochrome *b* sequences were obtained from GenBank®. The accession numbers are as follows: U70482, U74331-U74337, U74341, U74346-U74347, U74353, U74653, U74655. Note the sequence of *Pterodroma feae* is named in GenBank® as *P. deserta* (Penhallurick and Wink 2004). Cytochrome *b* sequence from a light-morph *P. arminjoniana* was kindly provided by Ruth M. Brown (unpubl. data). Tāiko haplotypes are as in chapter two.

Phylogenetic Analyses

Phylogenies were estimated by Bayesian inference with MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003) and maximum likelihood and maximum parsimony analyses using Paup* 4.0b10 (Swofford 2002). The HKY+I model of sequence evolution was selected using Modeltest 3.7 (Posada and Crandall 1998). A set of trees was constructed with 303bp of aligned DNA sequence. For Bayesian analysis, four chains were run for 6 million generations (temperature = 0.2); trees were sampled every 100 generations. Following a 20% burn-in a 50% majority rule consensus tree was constructed. A maximum likelihood tree was constructed with 1000 bootstrap replicates using a heuristic search with random sequence replicates and TBR branch swapping. Strict and 50% majority rule consensus maximum parsimony trees were constructed using a heuristic search with random sequence replicates. The same method was used for 150bp of cytochrome *b* sequence since only partial sequence was available (from GenBank®) for *Pseudobulweria rostrata* and *Pterodroma madeira*. All trees were rooted with the outgroup *Puffinus griseus.*

Results and Discussion

Phylogenetic analyses of the Magenta Petrel and *Pterodroma* petrels indicated the Magenta Petrel and the Chatham Island Tāiko are indistinguishable genetically. The Magenta Petrel grouped as a monophyletic clade with Tāiko in all trees (Bayesian inference, maximum likelihood and maximum parsimony) with strong posterior probability and bootstrap support (Fig. 7.3).

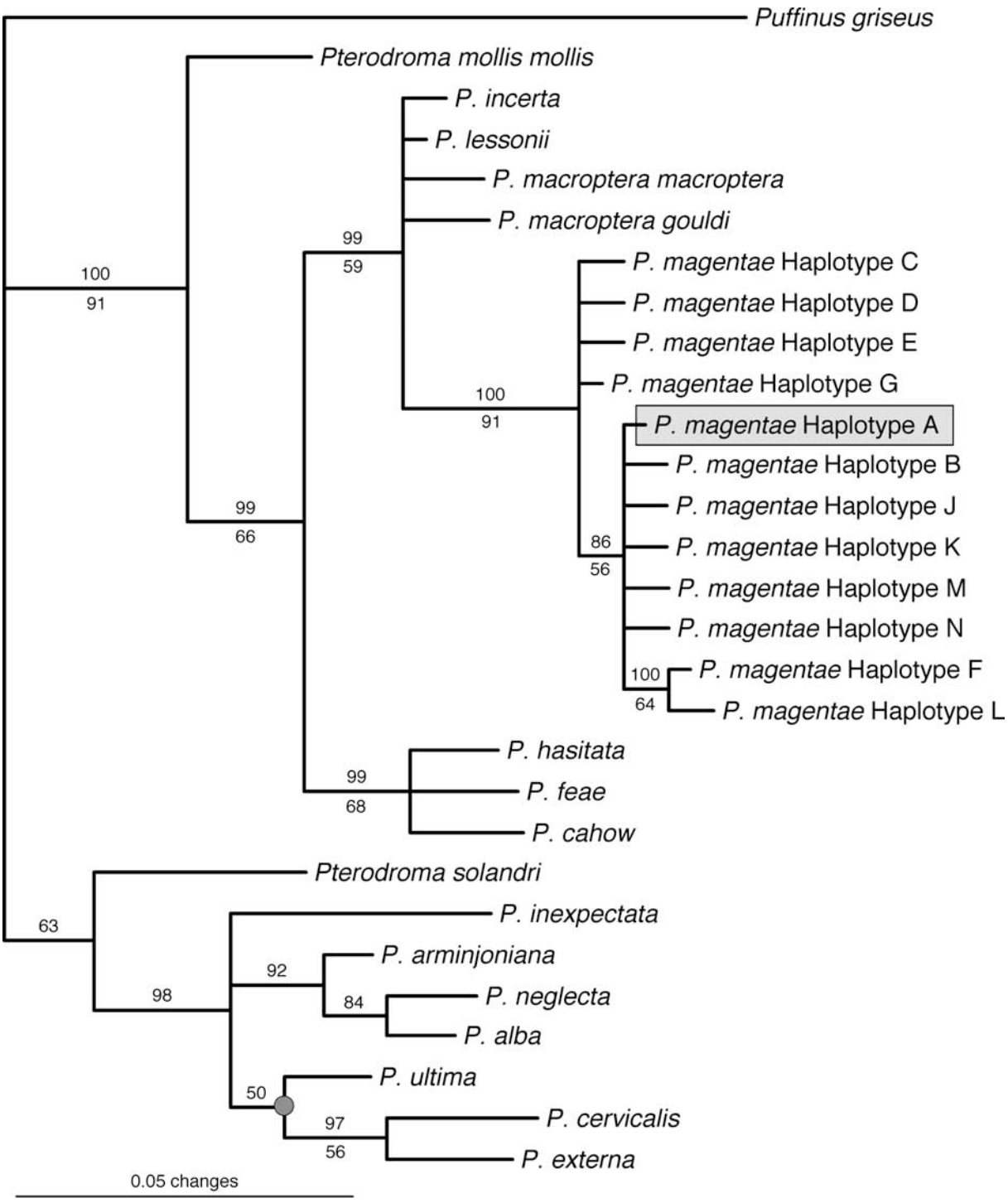

Figure 7.3 Phylogenetic relationship of the Magenta Petrel (haplotype A, highlighted) to *Pterodroma* petrels, based on mitochondrial cytochrome *b* sequence (303bp). The Bayesian inference consensus tree (shown) was inferred with the HKY+I model of sequence evolution. Bayesian inference posterior probabilities of clades have been converted to the percentage of trees with that topology and are presented above the branch; maximum likelihood bootstrap values are shown below the branch. Bayesian inference, maximum likelihood and maximum parsimony trees all concurred except where a grey circle indicates a node that occurred in both Bayesian inference and maximum likelihood trees, but not in the maximum parsimony tree.

The Magenta Petrel was collected in the central South Pacific Ocean and seen southeast of Easter Island/Rapa Nui and near the Juan Fernandez Islands, far from the Chatham Islands, 140 years ago (Giglioli and Salvadori 1869; Fig. 7.1). Interestingly, there have also been more recent reported sightings of the Tāiko off the coast of central Chile (Howell et al. 1996; Fig. 7.1). This raises the possibility that the Tāiko forages far into the South Pacific and/or that the species is not endemic to the Chatham Islands and may inhabit islands near South America. There are *Pterodroma* petrels whose foraging range extends from New Zealand to South American waters, including *P. lessonii*, *P. nigripennis* and *P. cookii* (Onley and Scofield 2007). Alternatively, an undiscovered form of the Tāiko may exist elsewhere (Taylor 2000). There are a number of *Pterodroma* with subspecies that breed on New Zealand islands and South American islands. These include the well-recognised subspecies of *P. neglecta neglecta* and *P. neglecta juana* and possible conspecific pairs of *P. pycrofti* and *P. longirostris* and *P. cervicalis* and *P. externa*. The Magenta Petrel has the same cytochrome *b* haplotype that is most common in living Tāiko (53% *N* = 90, chapter two) and in subfossil Tāiko bone samples (61% *N* = 44, chapter three). This would therefore suggest recent divergence or gene flow between the Chatham Island Tāiko population and any other putative South American population.

Morphological Similarity of the Tāiko and Pseudobulweria rostrata

The identification of petrel specimens using morphology alone is difficult as is demonstrated by an unexpected result of this study. DNA sequence obtained from a specimen labelled *Pseudobulweria rostrata* exactly matched the second most common haplotype found in the living Tāiko population (G/H/I, 17% *N* = 90) and Tāiko bones (23% *N* = 44, chapter three). Subsequent examination of the specimen by Paul Scofield at the Canterbury Museum verified that it was indeed a Tāiko (pers. comm.). The specimen was collected in the Pacific Ocean some time before 1910, when no other specimens of the Tāiko or *P. rostrata* were available to the Canterbury Museum for comparison. The finding that this specimen is in fact a Tāiko is significant. There are currently only two other known specimens of Tāiko worldwide, one a study skin of an adult (Imber 1998), the other a fledgling mounted for display (held at Te Papa Tongarewa, Wellington).

This study has shown that genetic analysis provides an important tool to identify individual specimens to the species level. Museum specimens for which identification analysis is required are typically old and contain degraded DNA. For this reason, ancient DNA techniques are required for their DNA extraction and amplification. Typically only short DNA fragments can be amplified (Mitchell et al. 2005). Fortunately, short DNA sequences are still

useful for specimen identification (Hajibabaei et al. 2006; Robins et al. 2007). Only partial cytochrome *b* sequence was available on GenBank® for *Pseudobulweria rostrata* and *Pterodroma madeira* and additional samples were unable to be obtained from these two species. Phylogenetic and morphological analyses show that *Pseudobulweria* species are not closely related to *Pterodroma* (Imber 1985; Bretagnolle et al. 1998; Penhallurick and Wink 2004). Furthermore, although Zino's Petrel *(Pterodroma madeira)* is within the same *Pterodroma* subgenus as the Tāiko it is not morphologically similar to the Magenta Petrel (Imber 1985; Onley and Scofield 2007). Phylogenetic analyses using 150bp of DNA sequence produced Bayesian inference, maximum likelihood and maximum parsimony trees that all excluded *Pseudobulweria rostrata* and *Pterodroma madeira* as being phylogenetically closely related to the Magenta Petrel (Fig. 7.4). These two species were subsequently excluded from the analyses using longer DNA sequence.

Conclusion

There are many islands off the coast of South America and these are not all well studied (Simeone et al. 2003). It is therefore possible for a breeding population of the Tāiko to survive undetected. If correct this would have major implications for the conservation of the Tāiko, the world's most endangered seabird species.

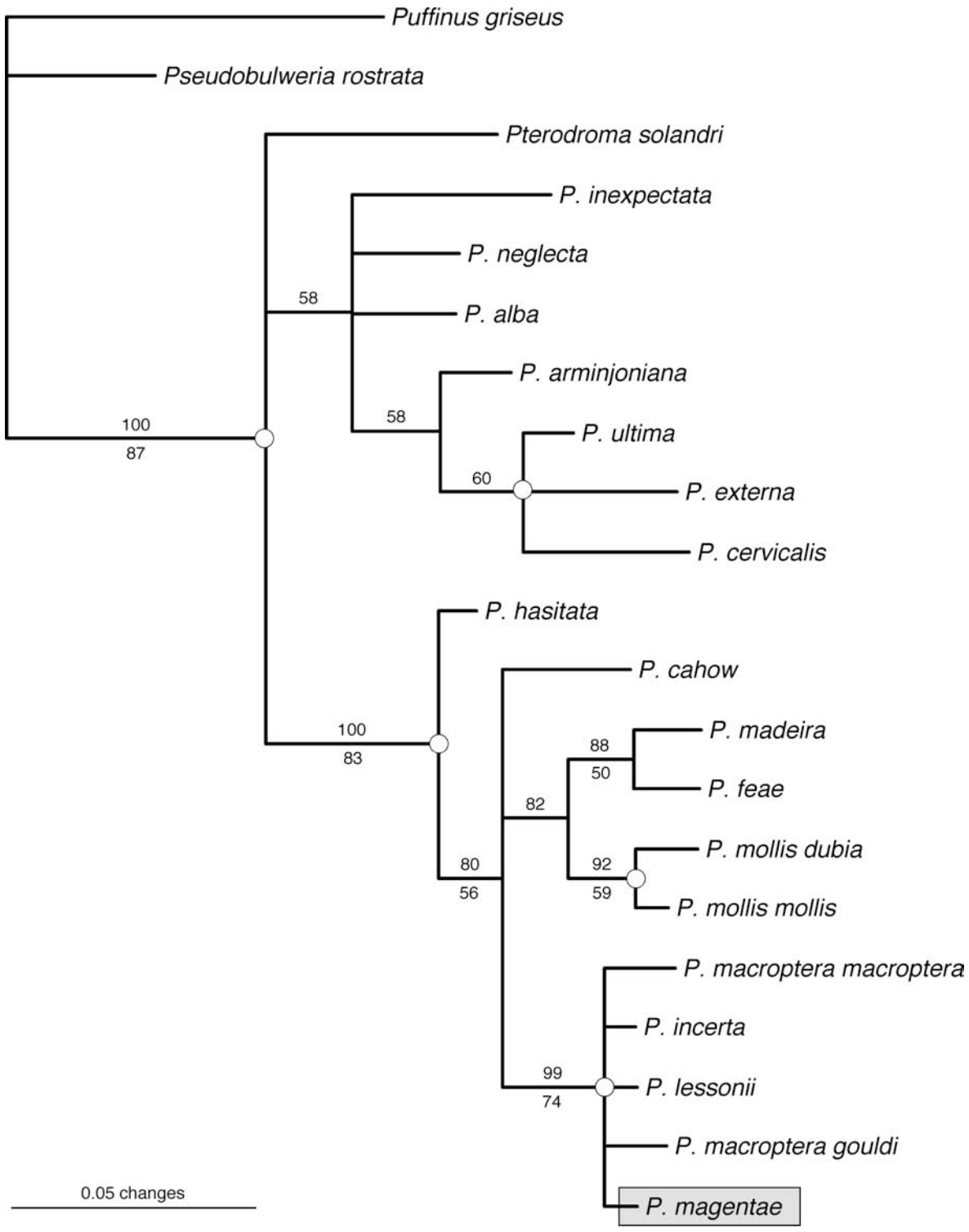

Figure 7.4 Phylogenetic relationship of the Magenta Petrel (highlighted) to *Pterodroma* and *Pseudobulweria* petrels, based on mitochondrial cytochrome *b* sequence (150bp). The Bayesian inference consensus tree (shown) was inferred with the HKY+I model of sequence evolution. Bayesian inference posterior probabilities of clades have been converted to the percentage of trees with that topology and are presented above the branch; maximum likelihood bootstrap values are shown below the branch. Bayesian inference and maximum likelihood trees concurred. White circles indicate nodes that also occurred in maximum parsimony trees.

Acknowledgements Mike Imber collected samples from the Magenta Petrel specimen with assistance from Claudio Pulcher and permission from the Museo Regionale di Scienze Naturali Torino. Graeme Taylor, Paul Scofield and the Canterbury Museum provided samples of other petrel specimens. Judith Robins replicated the extraction and amplification of Magenta Petrel DNA at the University of Auckland and Ruth M. Brown kindly provided unpublished sequence from a light-morph *P. arminjoniana*. Tamara Sirey, Leon Huynen and Jennifer Hay gave technical advice, Mike Imber, Paul Scofield, David Crockett and Graeme Taylor offered advice on petrel taxonomy. Vivienne Ward and Craig Millar provided assistance with Figure 7.1. The Alexander Turnbull Library gave permission for reproduction of the image of the *Magenta* ship and the British Library the plate of the Magenta Petrel.

References

Bourne WRP (1964) The relationship between the Magenta Petrel and the Chatham Island Tāiko. Notornis 11:139-144

Bretagnolle V, Attie C, Pasquet E (1998) Cytochrome-*b* evidence for validity and phylogenetic relationships of *Pseudobulweria* and *Bulweria* (Procellariidae). Auk 115:188-195

Crockett DE (1994) Rediscovery of Chatham Island Tāiko *Pterodroma magentae*. Notornis (Supplement) 41:49-60

Giglioli EH (1875) Viaggio Intorno al Globo Della R. Pirocorvetta Italiana Magenta. V. Maisner E Compagnia, Milano

Giglioli HH, Salvadori T (1869) On some new Procellariidae collected during a voyage round the world in 1865-68. Ibis 5:61-68

Godman FDC (1907-1910) A Monograph of the Petrels. Witherby & Co., London

Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PDN (2006) A minimalist barcode can identify a specimen whose DNA is degraded. Molecular Ecology Notes 6:959-964

Harrison P (1983) Seabirds An identification Guide. Reed, Beckenham

Howell SNG, Ainley DG, Webb S, Hardesty BD, Spear LB (1996) New information on the distribution of three species of Southern Ocean gadfly petrels (*Pterodroma* spp.). Notornis 43:71-78

Imber MJ (1985) Origins, phylogeny and taxonomy of the gadfly petrels *Pterodroma* spp. Ibis 127:197-229

Imber MJ, Tennyson AJD, Taylor GA, Johnston P (1998) A second intact specimen of the Chatham Island Tāiko *(Pterodroma magentae)*. Notornis 45:247-254

Kennedy M, Page RDM (2002) Seabird supertrees: combining partial estimates of Procellariifrom phylogeny. Auk 119:88-108

Mitchell D, Willerslev E, Hansen A (2005) Damage and repair of ancient DNA. Mutation Research 571:265-276

Nunn GB, Cooper J, Jouventin P, Robertson CJR, Robertson GG (1996) Evolutionary relationships among extant albatrosses (Procellariiformes: Diomedeidae) established from complete cytochrome-*B* gene sequences. Auk 113:784-801

Nunn GB, Stanley SE (1998) Body size effects and rates of cytochrome *b* evolution in tube-nosed seabirds. Molecular Biology and Evolution 15:1360-1371

Onley D, Scofield P (2007) Albatrosses, Petrels and Shearwaters of the World. Princeton University Press, Princeton

Penhallurick J, Wink M (2004) Analysis of the taxonomy and nomenclature of the Procellariiformes based on complete nucleotide sequences of the mitochondrial cytochrome *b* gene. Emu 104:124-147

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution. Bioinfomatics 14:817-818

Robins JH, Hingston M, Matisoo-Smith E, Ross HA (2007) Identifying *Rattus* species using mitochondrial DNA. Molecular Ecology Notes 7:717-729

Ronquist F, Huelsenbeck JP (2003) MRBAYES 3: Bayesian phylogenetic inference under mixed models. Bioinfomatics 19:1572-1574

Salvin O (1876) Critical notes on Procellariidae. Part 2. The new species of petrels obtained during the voyage of the Italian Corvette 'Magenta' round the world. Rowley's Ornithological Miscellany 4:249-257

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York

Seutin G, White BN, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69:82-90

Simeone A, Luna-Jorquera G, Bernal M, Garthe S, Sepulveda F, Villablanca R, Ellenberg U, Contreras M, Munoz J, Ponce T (2003) Breeding distribution and abundance of seabirds on islands off north-central Chile. Revista Chilena de Historia Natural 76:323-333

Swofford DL (2002) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4. Sinauer Associates, Massachusetts

Taylor GA (2000) Action Plan for Seabird Conservation in New Zealand Part A: Threatened Seabirds. Threatened Species Occasional Publication 16. Department of Conservation, Wellington http://www.doc.govt.nz/upload/documents/science-and-technical/TSOP16.pdf

Chapter Eight

General Discussion

Genetic research of rare and endangered species can be very beneficial to their conservation management. This is especially important when species are difficult to observe and study due to their location and life history characteristics. However, many endangered species have low genetic variation thereby limiting the variety and depth of genetic analyses that can be performed. As a population declines in size, genomic variation typically also declines. Sufficient variation may then be lacking to examine aspects of the biology of the species at a contemporary timeframe, as well as the species' history.

Fortunately, the Tāiko population has retained sufficient genetic variation for detailed genetic analyses to be possible. This provides an exceptional opportunity for an in-depth study of a quintessentially endangered species. The Tāiko is on the brink of extinction, with a population size that is orders of magnitude smaller than many other species also considered "critically endangered". These very low numbers allow detailed analyses involving almost every individual in the population using both genetic and observational methods. The combination of these two approaches is very powerful.

The high level of genetic variation found in Tāiko enables investigation into the population at different time frames. The history of the Tāiko population can be explored, not just by examining ancient DNA but also by inference from the patterns of variation in modern DNA. For some rare species only research using ancient DNA may be possible. By identifying relatedness between individuals, the population can be studied in a contemporary timeframe providing information that is directly applicable to current conservation management of the

species. The same genetic data can also be used to predict the future retention or loss of the variation.

It is unfortunate that the many avenues of investigation available to genetic research on Tāiko are not available to research on other endangered species such as the other iconic Chatham Island bird, the Black Robin. As technology and methods of analysis advance even more information could be gleaned from the genomes of rare species. It is therefore important that the genetic variation in Tāiko is conserved for future research that may be able to achieve even more towards saving this enigmatic bird from extinction.

Research Findings and Application to Conservation

This study illustrated that the adult Tāiko population retains a large proportion of the past mitochondrial variation. However, this high level of variation could be lost in just one generation from now since there is evidence of reduced genetic diversity in Tāiko chicks. This emphasises the importance of enhancing the productivity of pairs with rare mitochondrial DNA haplotypes and nuclear alleles. This finding is also relevant to the selection of Tāiko chicks to be translocated to the predator-exclusion site, to maintain genetic diversity and reduce founder effects in the future colony.

Mitochondrial DNA sequencing of Tāiko caught in flight and not associated with land indicated there are more Tāiko burrows yet to be found. It is imperative that these burrows are discovered so Tāiko adults and chicks can be protected from the major threat imposed by introduced predators. Individual Tāiko were identified using genetic analyses that would be of high priority for tracking. In addition, search areas could be recommended for specific birds, since Tāiko (especially males) were shown to be philopatric.

The more philopatric tendencies of males could be utilised in the transfer of chicks to the predator-exclusion site. Slightly more males than females may be transferred, as they are more likely to return to the site to breed. This conservation initiative is important since DNA sexing has identified an excess of unpaired Tāiko males. This is suggestive of the Allee effect that reduced density of potential mates is acting to decrease population productivity. Concentrating the new colony within a small area should help to overcome this effect.

Further knowledge of the Tāiko mating system and behaviour was gained using nuclear genetic markers. It is important for conservation that the biology of Tāiko is understood. Philopatry was evident in nuclear genetic markers but surprisingly none of the unpaired non-breeding males were offspring of known breeding pairs. In addition, more Tāiko caught in flight but not on land were identified as not having first order relatives in the population thereby expanding the number of potential undiscovered burrows thought to exist. It is even

possible that Tāiko could be found on islands as far away as South America. Phylogenetic analyses showed the Magenta Petrel is in fact a Tāiko. The Magenta Petrel was collected in the central South Pacific, and later seen near islands off the coast of Chile. These islands are not well studied, so the possibility that Tāiko might be found there is worth investigation.

Some Avenues for Future Research

Ancient DNA from petrel bones found on islands around South America could be examined to ascertain if a population of Tāiko ever bred (or could still be) in that area. In addition, petrel bones collected from islands around the main Chatham could be analysed at the molecular level to more accurately identify species. Museum specimens assigned to petrel species that are superficially similar to Tāiko could also be DNA sequenced to verify their identity. Since so few specimens of Tāiko exist, every specimen is important for both morphological analysis and public display.

Subfossil bones of an as yet undescribed *Pterodroma* have been found in the Chatham Islands that are very similar to Tāiko. These could be analysed genetically to determine if they are in fact from a species distinct from Tāiko and if so, the evolutionary relationship to Tāiko and other petrels.

Moriori middens could be excavated and bones analysed to examine past distribution of Tāiko and the possible transportation of Tāiko around the island. This could indicate if Tāiko food resources were shared or traded between different imi (tribes). In addition, middens found near rākau momori (Moriori tree carving) depictions of birds could be investigated to see if the carvings were intended as a sign indicating the location of Tāiko breeding colonies.

Birds of the genus *Pterodroma* are currently not well studied. As more genetic research is performed in these species, more comparisons can be made between species. For example, how does the level of genetic variation compare between Tāiko and other endangered *Pterodroma* and congeneric species that are not endangered? How are the mating systems different between Tāiko and *Pterodroma* that exist in numerous dense colonies? Is the rate of extra-pair paternity more common? Does egg-dumping and nest parasitism by females occur? Genetic research of *Pterodroma cookii, P. arminjoniana* and *P. cahow* is currently underway; it will be interesting to compare the results of these studies to those found for the Tāiko.

Genetic monitoring of Tāiko should continue and the practical conservation actions recommended in this thesis should be implemented. This is especially important with regards to any more Tāiko caught in flight. It will be a number of years before the new Tāiko colony at

the Sweetwater Covenant is established, however, in time, the genetic structure of this colony should be monitored for founder effects.

Technological advances could extend this study of the Tāiko population. As ancient DNA techniques develop, more variable markers and even whole mitochondrial genomes could be amplified and nuclear DNA could be more easily sequenced. This would allow more detailed analysis of past Tāiko populations such as their location and differentiation. New massively parallel sequencing methods will enable sequencing of whole mitochondrial genomes (both ancient and modern) to be much faster and substantially cheaper. A greater variety of mitochondrial DNA markers will therefore be available for analyses. In addition, large-scale sequencing of nuclear genomes, even from ancient DNA, is likely to be possible in the near future.

Appendix A

Supplementary Material for Chapter Two

Methods

The complete mitochondrial cytochrome *b* gene was amplified using the polymerase chain reaction (PCR) with primers L14863 5'-TTTGCCCTATCTATCCTCAT-3' (Nunn et al. 1996) and HTāikoThr2 5'-GGTTTTACAAGACCAATGTT-3' (designed by Leon Huynen). Reaction volumes totalled 50μl and included 1X PCR Buffer (Invitrogen), 1.5mM $MgCl_2$ (Invitrogen), 50μM of each dNTP (Bioline), 0.6μM of each primer (Invitrogen), 1 U of Platinum® Taq DNA polymerase (Invitrogen) and 50-200ng of DNA. A negative control (with no added DNA) was included in each set of PCRs. The following PCR profile was performed in an Applied Biosystems GeneAmp® PCR System 9700: 94°C for 2 min, then 30 cycles of 94°C for 30 sec, 59°C for 30 sec, 72°C for 30 sec and a final extension at 72°C for 5 min. The PCR products were then purified using a QIAquick® PCR Purification kit (Qiagen) or an ExoSAP method. The ExoSAP method (adapted from Amersham Biosciences') involved adding 20 units of exonuclease 1 ($usb_®$) and 4 units of shrimp alkaline phosphotase ($usb_®$) directly to the 50μl reaction after PCR. The reaction was then incubated at 37°C for 30 min, 80°C for 15 min and then cooled to 4°C. Both DNA strands were sequenced on an Applied Biosystems 3730 DNA analyser by the Allan Wilson Centre Genome Service, using the BigDye™ Terminator Version 3.1 Ready Reaction Cycle Sequencing kit (Applied Biosystems). Sequencing primers included L14863, HTāikoThr2 and internal primers LCytB432 5'-TGAGGACAAATATCATTCTGAGG-3' and HCytB571 5'-GGAAGGTGAGGTGGATTAAGG-3'.

The entire domain I of the mitochondrial control region (from the end of tRNAGlu to the start of the F box, as defined by Pereira et al. 2004) was initially amplified by PCR with primers LtRNAGluTāiko2 5'-AATTCCTGCTTGGCCTTTCT-3' and HCRPtb 5'-CTAGGGGTGTAGGGGGAAAG-3'. Reaction volumes and reagents were as above except 200μM of each dNTP and 2 U of Platinum® Taq DNA polymerase were used. The following conditions were applied for PCR in a BIO-RAD iCycler: 94°C for 2 min, then 30 cycles of 94°C

for 45 sec, 62°C for 45 sec, 72°C for 45 sec, then a final extension at 72°C for 5 mins. The PCR products were purified using a QIAquick® PCR Purification kit (Qiagen) then sequenced (as above) with primers LtRNAGluTāiko2 and HCRPtb. Sequencing electropherograms displayed double peaks at some nucleotide sites, suggesting the presence of heteroplasmy. Control region domain I was cloned using the TOPO TA Cloning® kit (Invitrogen), following the manufacturer's instructions. The initial PCR amplification conditions were the same as above, with the final extension temperature (72°C) extended to 20 mins. The transformation was performed in DH5α™-T1® One Shot® chemically competent cells. Plasmids were isolated using a Purelink™ Quick Plasmid Miniprep kit (Invitrogen), following the manufacturer's instructions. DNA was quantified on a NanoDrop® spectrophotometer, then 300ng was sequenced (as above) with 3.2pmol of M13 forward and reverse primers.

Cloning identified that two unique DNA sequences were amplified within individuals. This indicated the presence of a nuclear pseudogene or duplication. Duplication of the mitochondrial control region is known in a Procellariiform species (Abbott et al. 2005) so primers were designed with specificity to each sequence copy, designated fragments 1 and 2. Fragment 1 primers were: forward F1AF 5'- AATGGCCCATGTGCGGTTGT-3' and reverse F1AR 5'-TTAATAGGTTTTGGTACGATGA-3'. Fragment 2 primers were: forward F2AF 5'-AATGGTCTATGTGTGGGTGC-3' and reverse F2AR 5'-TTAATAGGTTTTGGTACGGTAG-3'. A long mtDNA PCR product of ~8kb was amplified using the Expand Long Template PCR System (Roche). Primers for amplification were L14863 and H12SPt 5'-GGGTGTATCGTGGGTTATCG-3'. Reaction volumes totalled 20μl and included 1X Expand Long Template Buffer 1 (1.75mM $MgCl_2$, Roche), 350μM of each dNTP (Bioline), 0.3 μM of each primer (Invitrogen), 1.5 U of Expand Long Template Enzyme mix (Roche) and up to 500ng of DNA. A negative control (with no added DNA) was included. The following PCR profile was performed in an Applied Biosystems GeneAmp® PCR System 9700: 93°C for 2 min, then 10 cycles of 93°C for 10 sec, 60°C for 30 sec, 68°C for 4 min, then 25 cycles of 93°C for 15 sec, 60°C for 30 sec, 68°C for 4 min + 20 sec each cycle, then a final extension at 68°C for 7 mins. The long mtDNA PCR product was used as a template for amplification and sequencing with primers LtRNAGluTāiko and HCRPtb (see above for conditions). The same double peaks were apparent. The long product was also used as template for amplification and sequencing with the sets of specific primers F1AF and F1AR, F2AF and F2AR and the specific forward primers (i.e. F1AF and F2AF) with the conserved reverse primer HCRPtb (conditions as above).

Each domain I control region sequence was amplified with the specific forward primer F1AF with HCRPtb and F2AF with HCRPtb for 117 Tāiko DNA samples. Reactions volumes totalled

20μl and included 1X PCR Buffer (Invitrogen), 1.5mM $MgCL_2$ (Invitrogen), 200μM of each dNTP (Bioline), 0.6 μM of each primer (Invitrogen), 1 U of Platinum® Taq DNA polymerase (Invitrogen) and 50-200ng of DNA. A negative control (with no added DNA) was included in each set of PCRs. The following PCR profile was performed in a BIO-RAD iCycler: 94°C for 2 min, then 30 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 30 sec, then a final extension at 72°C for 5 mins. Each fragment was purified and sequenced (as for cytochrome *b* above) with primer HCRPtb. The resulting sequence was 315 base pairs (bp) long for each of fragment 1 and fragment 2.

Sequences were aligned using Sequencher™ version 4.2.2 (GeneCodes). A Neighbour Net network for control region sequences was constructed in SplitsTree version 4.6 (Huson and Bryant 2006). The Neighbour Net method combines DNA sequences into clusters that are progressively larger and overlapping (i.e. agglomeration) to construct a network (Bryant and Moulton 2002).

Results and Discussion

Examination of nucleotide polymorphisms, base composition and inheritance indicates mitochondrial origin of the cytochrome *b* and control region sequences. For cytochrome *b*, there were no length mutations, stop codons or frameshift mutations. All but one of the 12 substitutions were transitions, 10 out of 12 were in the third position of the codon. Two nucleotide substitutions resulted in two amino acid replacements. There is a bias against codons ending in G and T, and very low G content overall (12.7%). For these reasons, it is assumed the sequences amplified are mitochondrial in origin (Desjardins and Morais 1990; Sorenson and Quinn 1998; Bensasson et al. 2001).

For the control region sequences, heteroplasmy was apparent as double peaks at some nucleotide sites in electropherograms. Cloning identified that two unique sequences were amplified in single individuals. The differences between the sequences were so marked, that primers could be designed to amplify the sequences separately. Both sequences (fragments 1 and 2) were amplified within a long (~8kb) PCR product spanning part of tRNAGlu, the entire control region, tRNAPhe and part of 12SrRNA. The largest nuclear mitochondrial pseudogene found in the chicken genome is 1.7kb (Pereira and Baker 2004), so the ~8kb product amplified is mitochondrial in origin (Sorenson and Quinn 1998; Bensasson et al. 2001). Other evidence to support mitochondrial origin includes the fact that all 22 chicks (both male and female) sequenced for fragment 1 and 10 sequenced for cytochrome *b*, had the

same haplotype as their mother. There is also low G content in the light strand, 14.3% in fragment 1 and 14.9% in fragment 2, which is characteristic of mtDNA (Desjardins and Morais 1990).

Duplicated control regions have been identified in a number of organisms including avian species and, more specifically, seabird species (e.g. Abbott et al. 2005). The two fragments sequenced in Tāiko aligned easily and are equal in length (315bp). A Neighbour Net Network (Fig. A.1) showed clustering of sequences into two groups corresponding to the different (orthologous) fragments, rather than paralogous sequences (from the same individuals) clustering together. This suggests a pre-speciation duplication event and that the fragments are evolving independently (Abbott et al. 2005).

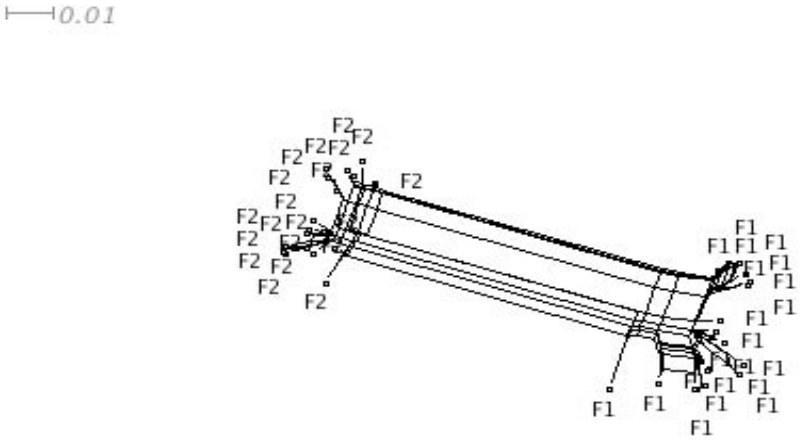

Figure A.1 Neighbour net network for fragment 1 (F1) and fragment 2 (F2) mitochondrial control region haplotypes in Tāiko *Pterodroma magentae* (constructed in SplitsTree version 4.6)

References

Abbott C, Double MC, Trueman JWH et al (2005) An unusual source of apparent mitochondrial heteroplasmy: duplicate mitochondrial control regions in *Thalassarche* albatrosses. Molecular Ecology 14:3605-3613

Bensasson D, Zhang D-X, Hartl DL, Hewitt GM (2001) Mitochondrial pseudogenes: evolution's misplaced witness. Trends in Ecology and Evolution 16:314-321

Bryant D, Moulton V (2002) NeighborNet: an agglomerative method for the construction of planar phylogenetic networks. In: Guigo R and Gusfield D (eds) Algorithms in Bioinformatics: WABI 2002, volume LNCS 2452. Springer, Berlin Heidelberg, pp375-391

Desjardins P, Morais R (1990) Sequence and gene organization of the chicken mitochondrial genome a novel gene order in higher vertebrates. Journal of Molecular Biology 212:599-634

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. Molecular Biology and Evolution 23:254-267

Nunn GB, Cooper J, Jouventin P (1996) Evolutionary relationships among extant albatrosses (Procellariiformes: Diomedeidae) established from complete cytochrome-*B* gene sequences. Auk 113:784-801

Pereira SL, Baker AJ (2004) Low number of mitochondrial pseudogenes in the chicken *(Gallus gallus)* nuclear genome: implication for molecular inference of population history and phylogenetics. BMC Evolutionary Biology 4: 17-24

Pereira SL, Grau ET, Wajntal A (2004) Molecular architecture and rates of DNA substitutions of the mitochondrial control region of cracid birds. Genome 47:535-545

Sorenson MD, Quinn TW (1998) Numts: a challenge for avian systematics and population biology. Auk 115:214-221

Appendix B

Supplementary Material for Chapter Three: Tāiko Subfossil Bones

Island Searches for Tāiko Remains

Only three bones identified as Tāiko have been discovered outside the main Chatham Island/Rēkohu/Wharekauri. These bones were found on nearby Pitt Island/Rangiauria (A. J. Tennyson in Aikman et al. 2001; Fig. B.1). The three Tāiko bones were found in an assemblage of around 10 000 bones, so it is likely that these were vagrant birds (A. J. Tennyson pers. comm.). Well-preserved bones of other petrels were found, so there was no preservation bias (A. J. Tennyson pers. comm.). Tāiko bones were not found in deposits on Mangere Island (Tennyson and Millener 1994), Little Mangere/Tapuaenuku or Rangatira/South East Island (Millener in Grant 1994; Fig. B.1). However, the islands (except for Chatham/Rēkohu/Wharekauri, Pitt/Rangiauria and Mangere) lack suitable conditions for bone preservation (Millener 1999). Searches for signs of living Tāiko were unsuccessful on Rabbit Island/Wharekaikite Motu (Imber and Lovegrove 1982), Rangatira/South East Island, Star Keys/Motuhope, Mangere Island and Houruakopara (Imber 1976; Fig. B.1). Antipodes Island was searched extensively for Tāiko bones without success (M. J. Imber pers. comm.; Fig. B.1).

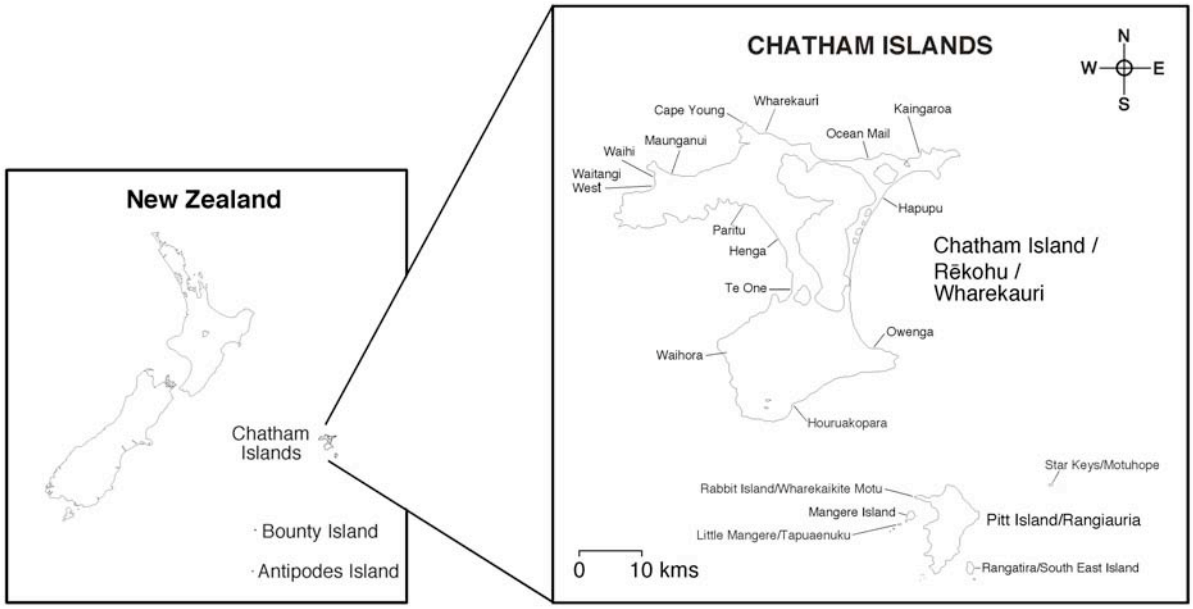

Figure B.1 Locations of searches for Tāiko bones / living birds

Tāiko Bones Collected from Natural Deposits

In this study, Paul Scofield and I collected Tāiko bones from the surface of sand dunes at eight locations around main Chatham Island (Table B.1, Fig. B.1). Sand dunes at Wharekauri and Hapupu beach were also searched, but no Tāiko bones were found. Marram grass *(Ammophila arenaria)* had over-grown the dunes at some locations (including Wharekauri, Ocean Mail, Maunganui and Hapupu beach) making it difficult to find bones. We found only one natural deposit at Owenga, but there were extensive middens. We purposefully avoided midden sites, so it was difficult to find bones at Owenga. Tāiko bones were often found in deposits with lots of land snail shells, suggesting the area was forested in the past. Good deposits were found where the side of a dune had been eroded exposing a face. Subsequent to this study, Tāiko bones were returned to the Chatham Islands as requested by imi / iwi authorities and the Department of Conservation. Additional bones collected from sand dunes were obtained from the osteological collection of the Canterbury Museum, Christchurch, New Zealand (Table B.2, Fig. B.1).

Table B.1 Subfossil bones collected in this study

Collection Location	Number of Bones Collected	Number of Bones with Successful DNA Sequencing
Te One	30	13
Henga	13	1
Paritu	7	4
Waitangi West	23	10
Maunganui Dunes	2	0
Wharekauri	0	-
Ocean Mail	1	0
Hapupu Beach	0	-
Hapupu (inland dunes)	5	0
Owenga	1	0
TOTAL	**82**	**28**

Note: 'Success' is defined here as having obtained sequences of all three Cytochrome *b* regions conforming to the requirements of multi-PCR amplifications outlined in the methods section of chapter three

Table B.2 Subfossil bones from the Canterbury Museum, Christchurch, New Zealand

Bone Identification Number	Collection Location	Date	Collector/s	Cytochrome *b* Haplotype
AV10, 986 K	Kaingaroa	1952	JR Eyles	Novel (K)
AV11, 339 O	Owenga	1962	JR Eyles	A
AV26, 463	Wharekauri	1972	B Norris	*(Puffinus griseus)*
AV28, 522 A	Cape Young	1974	RJ Scarlett et al.	*(P. griseus)*
AV28, 522 B	Cape Young	1974	RJ Scarlett et al.	*(P. griseus)*
AV29, 574 A	Waihi Dunes	1975	RJ Scarlett & HG Royds	-
AV29, 617 A	Maunganui Dunes	1975	RJ Scarlett & HG Royds	-
AV29, 617 B	Maunganui Dunes	1975	RJ Scarlett & HG Royds	A
AV29, 617 C	Maunganui Dunes	1975	RJ Scarlett & HG Royds	B
AV29, 617 D	Maunganui Dunes	1975	RJ Scarlett & HG Royds	A

Tāiko Bones Collected from Midden Deposits

A large archaeological study at Waihora in southwest Chatham uncovered Tāiko bones of midden origin (Sutton 1979a,b; Millener 1999; Fig. B.1). Tāiko bones were collected from three main sites within 2km of each other: Waihora (N.Z.A.A. Site C240/283), CHA (C240/681) and CHB (C240/680). The Waihora site (WH, C240/283) had a thin midden layer that was on top of an ancient sand dune (Sutton 1976). Some of the bones collected from Waihora (of assumed midden origin) were actually from older, natural deposits (Millener 1999). Material collected from the CHA and CHB sites however, was from authentic cultural deposits (Millener 1999). The Tāiko bone samples from Waihora used in this study had a similar appearance to the other midden bones, having broken ends, so are probably midden in origin. Tāiko bones of midden origin used in this study are outlined in Table B.3.

Table B.3 Subfossil bones from the Otago Museum, Dunedin, New Zealand

Archaeological Site	Bone Identification Label	Cytochrome *b* Haplotype
CHB/I/9 L.1	OA	A
CHB/I/14 L.1	OB	G/H/I
	OC	A
CHB/II/2 L.1	OD	G/H/I
CHB/II/17 L.1	OE	G/H/I
	OF	A
CHB/II/18 L.1	OG	A
	OH	G/H/I
WH/Vb/1 4	OI	A
	OJ	A
	OK	A
	OL	-

References

Aikman H, Davis A, Miskelly C, O'Conner S, Taylor GA (2001) Chatham Island Tāiko Recovery Plan 2001-2011. Threatened Species Recovery Plan 36. Department of Conservation, Wellington
http://www.doc.govt.nz/upload/documents/science-and-technical/TSRP36.pdf

Grant A (1994) Chatham Island Tāiko Recovery Plan 1994-2000. Department of Conservation, Wellington

Imber MJ (1976) The Tāiko. Wildlife a Review 7:35-40

Imber MJ, Lovegrove TG (1982) Leach's Storm Petrels *(Oceanodroma l. leucorhoa)* prospecting for nest sites on the Chatham Islands. Notornis 29:101-108

Millener PR (1999) The history of the Chatham Islands' bird fauna of the last 7000 years - a chronicle of change and extinction. Smithsonian Contributions to Palaeobiology 89:85-109

Sutton DG (1976) Radiocarbon dates from the Waihora mound site, south-west coast, Chatham Islands. New Zealand Archaeological Association Newsletter 19:195-196

Sutton DG (1979a) Island and coastal fowling strategies of the prehistoric Moriori. In: Birds of a Feather Osteological and Archaeological Papers from the South Pacific in Honour of R. J. Scarlett (ed. Anderson A). British Archaeological Reports, Oxford

Sutton DG (1979b) Polynesian Coastal Hunters in the Subantarctic Zone: A Case for the Recognition of Convergent Cultural Adaptation. PhD Thesis, Department of Anthropology, University of Otago, Dunedin

Tennyson AJD, Millener PR (1994) Bird extinctions and fossil bones from Mangere Island, Chatham Islands. Notornis (Supplement) 41:165-178

Appendix C

Supplementary Material for Chapter Three: Technical Aspects of Ancient DNA Research

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Ancient DNA (aDNA) Degradation and Damage

DNA damage was considered in the Tāiko aDNA study to prevent possible incorrect conclusions derived from sequencing artefacts. Sequencing of products from multiple PCRs allowed identification of artefacts caused by miscoding lesions. Over all successful samples, the rate of miscoding lesions was 6.2×10^{-3} per nucleotide site (1 miscoded lesion for every 161 bases). Of the 83 lesions detected, the vast majority (98%) were type II. This is consistent with theory (Lindahl 1993) and results from other ancient DNA studies (e.g. Gilbert et al. 2007). Type II transitions (C→T/G→A) are predominantly caused by hydrolytic deamination of cytosine to uracil and its analogues on either strand (but the mechanism for G→A transitions remains unknown, Lindahl 1993; Gilbert et al. 2007). Type I transitions (A →G/T→C) are much less common and could occur when adenine is deaminated to form hypoxanthine (Lindahl 1993; Hansen et al. 2001). In this study, only one type I transition (T→C) was observed as a miscoding lesion. This result is consistent with recent research that has found very few or no type I transitions in amplified ancient DNA, in contrast to earlier studies (Stiller et al. 2006; Gilbert et al. 2007). In this study, a single transversion lesion was also observed (C→A). This could be caused by DNA polymerase error during early amplifications of PCR due to few initial template molecules (Gilbert et al. 2007). Both of these non-type II coding errors occurred in the same bone sample that also had a high number of type II lesions. DNA from this sample was therefore highly damaged and probably had low template molecule concentration available for PCR. To ensure novel haplotypes identified in this study were not mistakenly defined due to aDNA damage, sequences were checked by multiple PCR amplifications.

aDNA Preservation

In this study, the overall success rate for aDNA extraction, amplification and sequencing at all three cytochrome *b* regions was 42%. This rate is similar to other aDNA studies (Stiller et al. 2006 and references therein). Tāiko DNA was successfully sequenced from almost every bone obtained from midden sites (92%, $n = 12$). The single bone from which aDNA could not be amplified was dark brown in colour. This may indicate high humic acid content that can inhibit PCR (Burger et al. 1999). The success rate for bones collected from sand dunes was much lower however (36%, $n = 92$).

Gilbert et al. (2003) found a significant correlation between DNA damage and archaeological site (rather than age of sample). It is interesting to note that none of the Tāiko bones from the Waihora middens had detectable miscoding lesions, whereas at least one sample from every dune location did (the exception being Kaingaroa which was only represented by a single bone). Ancient DNA was successfully extracted from Kiore (Pacific rat, *Rattus exulans*) bones from the same archaeological middens at Waihora (Matisoo-Smith et al. 1999). The PCR amplification success rate was 97% for 31 Kiore bones, suggesting these sites are good for preservation of aDNA (Robins et al. 2001). This agrees with the finding that aDNA is better preserved in the Tāiko bones from the Waihora middens than those collected from sand dunes.

For some Tāiko bone samples only a short DNA sequence could be obtained. Searches of GenBank® matched the short sequence from nine samples to the Titi/Sooty Shearwater *(Puffinus griseus)* and sequences from 12 samples matched the Little Blue Penguin/Korora *(Eudyptula minor)*. Therefore, it seems likely that some of the bones with poor amplification results were misidentified as Tāiko. Petrel bones can be difficult to accurately identify to species level (Warham 1990; Worthy 1999), especially when immature (Bourne 1967). This suggests that not all amplification failure was due to poor preservation of aDNA in the bones.

Reliability of Results

A checklist of criteria to reduce risks of incorrect results and contamination in ancient DNA research has been suggested (Willerslev and Cooper 2005). Rather than strictly adhering to each of these criteria it is logical to assess the reliability of studies on their individual situations and merits (Paabo et al. 2004). Protocols for animal studies need not be as stringent as those for human DNA research, because of the much lower risk of contamination (Gilbert et al. 2005). For example, analysis of DNA survival using methods other than PCR is of concern for ancient human and microbial research, but not necessary for this study. The Tāiko bone samples were not at risk of contamination from handling or the environment. Contamination

risk for these samples would have come from PCR products. For this reason, DNA extraction and PCR set up was performed in a dedicated aDNA laboratory, in a separate building from the laboratory where extraction of DNA from modern samples and PCR occurs. Access to the aDNA laboratory was controlled, with only 'one-way traffic' permitted for people and samples, reagents and equipment. That is, movement from ancient to modern laboratory; never the reverse. Surfaces, pipettes and other implements were cleaned with 10% sodium hypochlorite then 80% ethanol before use. DNA extraction and pre-PCR set up were conducted in separate rooms. The bench used for PCR set up was UV-irradiated each night. Contamination was monitored using extraction and PCR negative controls. In addition, independent replication of aDNA extraction and amplification of some samples was achieved by another researcher at a different institution.

Willerslev and Cooper (2005) suggest cloning of amplification products is essential in assessment of contamination, DNA damage and discovery of nuclear insertions (numts). There is only a low risk of contamination for this study because the species of interest is a wild bird (Gilbert et al. 2005). No numts were detected in the mitochondrial DNA sequences obtained from the modern Tāiko population (appendix A). The close homology of the ancient Tāiko DNA sequences suggests that they are not numts either. For high confidence in sequence accuracy, Bower et al. (2005) recommend sequencing 20 clones per PCR product. This would have been very time-consuming and expensive for this project. Furthermore, it may be impossible to completely remove the effects of aDNA damage using this strategy (Ho et al. 2007). A more economic approach is to verify sequences by multiple independent amplifications (Paabo et al. 2004). Even in the unlikely situation of only one initial template molecule being available for each PCR, the chance of two independent sequences having the same miscoding lesion is very small (Hofreiter et al. 2001). DNA damage was therefore identified and assessed in this study using multiple PCR amplifications; cloning was not necessary. This multi-PCR strategy also rendered uracil-N-glycosylase (UNG) treatment unnecessary. Although UNG can remove deaminated cytosine products (Lindahl 1993), it can cause strand-breaks in a significant proportion of the DNA molecules when concentration is low and is not necessary when concentration is high (Hofreiter et al. 2001).

Since almost every known living adult Tāiko has been sequenced for cytochrome *b*, this prior knowledge allows assessment of the ancient DNA results that would be impossible for studies of extinct animals. The same methodological criteria were therefore not necessary.

References

Bourne WRP (1967) Subfossil petrel bones from the Chatham Islands. Ibis 109:1-7

Bower MA, Spencer M, Matsumura S, Nisbet RER, Howe CJ (2005) How many clones need to be sequenced from a single forensic or ancient DNA sample in order to determine a reliable consensus sequence? Nucleic Acids Research 33:2549-2556

Burger J, Hummel S, Herrmann B, Henke W (1999) DNA preservation: a microsatellite-DNA study on ancient skeletal remains. Electrophoresis 20:1722-1728

Gilbert MTP, Bandelt H-J, Hofreiter M, Barnes I (2005) Assessing ancient DNA studies. Trends in Ecology and Evolution 20:541-544

Gilbert MTP, Binladen J, Miller W, Wiuf C, Willerslev E, Poinar H, Carlson JE, Leebens-Mack JH, Schuster SC (2007) Recharacterization of ancient DNA miscoding lesions: insights in the era of sequencing-by-synthesis. Nucleic Acids Research 35:1-10

Gilbert MTP, Hansen AJ, Willerslev E, Rudbeck L, Barnes I, Lynnerup N, Cooper A (2003) Characterization of genetic miscoding lesions caused by postmortem damage. American Journal of Human Genetics 72:48-61

Hansen AJ, Willerslev E, Wiuf C, Mourier T, Arctander P (2001) Statistical evidence for miscoding lesions in ancient DNA templates. Molecular Biology and Evolution 18:262-265

Ho SYW, Heupink TH, Rambaut A, Shapiro B (2007) Bayesian estimation of sequence damage in ancient DNA. Molecular Biology and Evolution 24:1416-1422

Hofreiter M, Jaenicke V, Serre D, von Haeseler A, Paabo S (2001) DNA sequences from multiple amplifications reveal artefacts induced by cytosine deamination in ancient DNA. Nucleic Acids Research 29:4793-4799

Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362:709-715

Matisoo-Smith E, Sutton DG, Ladefoged TN, Lambert DM, Allen JS (1999) Prehistoric mobility in Polynesia: mtDNA variation in *Rattus exulans* from the Chatham and Kermadec Islands. Asian Perspectives 38:186-199

Paabo S, Poinar H, Serre D, Jaenicke-Despres V, Hebler J, Rohland N, Kuch M, Krause J, Vigilant L, Hofreiter M (2004) Genetic analyses from ancient DNA. Annual Review of Genetics 38:645-679

Robins JH, Matisoo-Smith E, Furey L (2001) Hit or miss? Factors affecting DNA preservation in Pacific archaeological material. In: Australian Connections and New Directions: Proceedings of the 7th Australasian Archaeometry Conference (eds. Jones M, Sheppard P). Department of Anthropology, University of Auckland

Stiller M, Green RE, Ronan M, Simons JF, Du L, He W, Egholm M, Rothberg JM, Keates SG, Ovodov ND, Antipina EE, Baryshnikov GF, Kuzmin YV, Vasilevski AA, Wuenschell GE, Termini J, Hofreiter M, Jaenicke-Despres V, Paabo S (2006) Patterns of nucleotide misincorporations during enzymatic amplification and direct large-scale sequencing of ancient DNA. Proceedings of the National Academy of Science USA 103:13578-13584

Warham J (1990) The Petrels Their Ecology and Breeding Systems. Academic Press, London

Willerslev E, Cooper A (2005) Ancient DNA. Proceedings of the Royal Society B 272:3-16

Worthy TH (1999) What was on the menu? Avian extinction in New Zealand. New Zealand Journal of Archaeology 19:125-160

Appendix D

Genetic Data

Table Key

C = Chick

NB = Non-breeder

B = Breeder

L = Caught at lights only (status not known)

Lights = Tuku light site unless otherwise stated

ml = amount of whole blood added to lysis buffer (in millilitres)

Taiko *(Pterodroma magentae)* Genetic Data								Mitochondrial DNA Haplotypes			Microsatellite Genotypes							
Band Number	**Sample Number**	**Status**	**Location**	**Date**	**Sex**		**Comment**	**Cyt *b***	**F1 CR**	**F2 CR**	**Tch6**	**Paequ3**	**Paequ8**	**Paequ13**	**RBG18**	**Tch25**	**RBG29**	**De33 (Sex-linked)**
Unbanded	T204 (embryo)	Embryo	S29	1/4/00	M		Dead chick in egg		F		-	-	-	-	-	-	-	-
E107404	T304	C, B	S40	4/11/99	F		N1 chick 1991, S40 from 1999	C	C	C	160/160	232/234	229/229	159/159	179/179	228/228	143/145	134
E107406	T126 (0.1ml) T127 (few drops) T128 (0.4ml)	B	S4	1/10/97	M		S4 from 1992	G	K	M	160/164	236/238	229/229	159/161	179/179	224/224	141/143	128/128
E107408	T302 (0.15ml)	C, B	S29, S31	11/10/99	M		S4 chick 1993, Lights & S29 1999, S31 from 2001		S	S	160/160	236/242	229/229	159/161	179/179	224/228	141/143	128/128
E107409	T124 (0.4ml) T125 (0.25ml)	B	S4	1/10/97	F		S4 from 1993	A	S	S	160/160	240/242	229/229	159/159	179/179	226/228	141/145	128
E107410	T300 (0.15ml) T1032 (0.05ml) T1033 (0.15ml)	B	S19	1/10/99 1/10/06 1/10/06	M		Lights 1993, S19 from 1998	A	Y	Y	160/160	240/242	229/229	159/159	179/181	224/224	143/147	128/134
E107411	T55	B	S18	1/10/96	F		Lights & S18 from 1993	B	B	B	160/160	236/238	229/231	159/159	179/179	224/228	143/145	128
E107414	T54	B	S19	1/10/96	F		Lights 1993, S19 from 1996	A	V	Q	160/160	228/238	229/229	161/163	179/179	224/226	143/147	128
E107415	SB15 (0.4ml)	B	S46, 25-14	6/10/02	F		Lights 1993, S46 2002, 25-14 from 2003	G	K	M	160/160	232/238	229/229	159/159	179/179	224/228	143/145	128
E107416	T812	L	Lights only	24/10/01	F		Lights 1993 & 2001 Timihonga close to 251 (unconfirmed)	A	Z	Z	160/160	234/236	229/229	159/163	179/179	224/228	145/147	134
E107417	T809	B	many burrows, S71	21/10/01	M		Lights 1993, near S55 1999, S44 2001, S50 2002 & 2003, S71 from 2004	A	W	W	160/160	236/250	229/229	159/161	179/179	224/224	143/147	128/134
E107419	T1028 (0.25ml)	C, NB	S3	1/10/04	M		S1 chick 1995, S3 from 2004		L	M	160/160	234/238	229/229	159/159	179/181	228/228	143/143	128/134
E107421	E06	C	S19	1/4/97	F						-	-	-	-	-	-	-	-
E107422	D01, D02	C	S10	1/4/97	M			B	B		160/160	238/242	229/229	159/161	179/179	228/228	143/145	128/134
E107423	T95 T1013 (0.4ml)	C, B	S4	1/4/97	M		24 chick 1997, S4 from 2004		S	S	160/160	236/242	229/229	159/159	179/179	224/228	141/143	128/128
E107424	T100	C	S1	1/4/97	F			G			160/160	234/234	229/229	159/159	179/181	224/228	143/143	134
E107425	T101 (0.4ml)	L	Lights only	20/10/97	M		Interested in area opposite Telemetry Hill, landed near S26?	A	U	U	160/164	232/238	229/229	159/161	179/179	228/228	143/145	134/134
E107426	T102 (0.4ml)	L	Lights only	20/10/97	M		Lights 1997	A	X	X	160/160	228/238	229/229	159/159	179/179	224/228	143/143	128/128
E107427	T103 (0.25ml)	NB	near N1	21/10/97	M		Lights 1997 then found on surface 1km south of N1	C	C	C	160/160	236/238	229/229	159/159	179/179	224/228	143/145	134/134

Taiko *(Pterodroma magentae)* Genetic Data								Mitochondrial DNA Haplotypes			Microsatellite Genotypes							
Band Number	**Sample Number**	**Status**	**Location**	**Date**	**Sex**		**Comment**	**Cyt *b***	**F1 CR**	**F2 CR**	**Tch6**	**Paequ3**	**Paequ8**	**Paequ13**	**RBG18**	**Tch25**	**RBG29**	**De33 (Sex-linked)**
E107428	T104 (0.4ml)	?	Near Lake Te Rangatapu	22/10/97	F		Lights 1997, Otawae lights 2003 tracked to burrow on south-east shore of Lake Te Rangatapu	D	D	D	160/160	232/236	229/229	159/159	179/179	224/228	145/147	128
E107429	T105 (0.4ml)	B	S25, S48	29/10/97	M		Lights 1997, S25 then S48 from 2001	A	Y	Y	160/160	240/242	229/231	159/161	179/181	224/224	143/145	128/134
E107430	T109	B	many burrows, S40	21/11/97	M		Lights 1997, S34/S33/S43 1999, S40 from 2002	E	E	E	160/160	232/234	229/229	159/159	179/179	224/224	143/145	128/134
E107431	T110	L	Lights only	24/11/97	M		Lights 1997 & 2003	F	F	F	160/160	232/238	229/229	159/159	179/179	228/228	143/145	128/134
E107432	T111	L	Lights only	24/11/97	F		Lights 1997	G	K	M	160/160	232/234	229/229	159/159	179/179	224/224	145/145	128
E107433	T301 (0.15ml)	NB	near S71	10/10/99	M		Lights 1999, near S71 2001	A	V	Q	160/160	238/242	229/229	159/159	179/179	228/228	143/145	128/134
E107434	T303 (0.4ml)	B	S35	18/10/99	F		Lights 1999, S35 from 2001				-	-	-	-	-	-	-	-
E107435	T306	L	Lights only	8/11/99	M		Lights 1999	A	Z	Z	160/160	228/240	229/229	159/159	179/179	224/224	143/145	128/134
E107436	T307 (0.22ml)	NB	below S35	12/11/99	M		Lights 1999, below S35 2000, Otawae lights 2003	A	Y	Y	160/160	240/240	229/231	159/159	179/179	224/228	143/147	128/128
E107437	T308 (0.44ml)	B	S45, 25-14	13/11/99	M		Lights 1999 & 2001, S45 1999, 25-14 from 2003	A	Y	Y	160/160	228/240	229/229	161/163	179/179	224/224	143/147	128/134
E107438	T200 (0.25ml)	C, NB	S79	8/4/00	M		S19 chick 2000, Lights 2003, S79 from 2006		V	Q	160/160	238/242	229/229	159/161	179/181	224/226	143/147	128/128
E107439	T202 (0.25ml)	C	S46	8/4/00	M			G	K		160/160	232/240	229/229	159/159	179/181	224/224	145/147	128/128
E107440	T152 (0.15ml)	C	S35	8/4/00	M			A	T	T	160/160	238/250	229/229	159/159	179/181	224/224	145/147	128/128
E107441	T153 (0.35ml)	C, NB	S77	1/5/00	M		S53 chick 2000, Lights 2003 & 2005, S77 from 2006	A	Y	Y	160/160	240/242	229/231	161/163	179/179	224/224	143/145	128/134
E107442	T154 (0.4ml)	C	S55	1/5/00	F				V		160/160	232/238	229/229	159/159	179/179	224/224	145/147	128
E107443	T155 (0.05ml)	C	S1	1/5/00	M			G			160/160	232/242	229/231	159/163	179/181	224/228	143/143	128/128
E107444	T604 (0.15ml)	NB	S49, S46	1/11/00	M		S49 from 2000, also S46 2006	A	Y	Y	160/160	240/242	229/229	159/163	179/179	224/224	145/147	128/134
E107445	T605 (0.15ml)	B	S48	1/11/00	M		S48 from 2003	G	K	M	160/160	238/240	229/229	159/159	179/179	224/224	143/147	128/128
E107446	T607 (0.15ml)	B	S50	9/11/00	M		S50 from 2000	A	U	U	160/160	234/242	229/229	159/161	179/179	224/228	143/145	134/134
E107447	T701	B	S55	14/1/01	M		S55 from 2001	D	D	D	160/160	232/232	229/229	159/159	179/179	224/228	145/147	128/134
E107448	T702	B	S29	15/1/01	M		S29 from 2001	F	F	F	160/160	232/238	229/229	159/163	179/181	224/228	143/145	134/134
E107449	T703	B	S18	17/1/01	F		S18 from 2001	G	K	M	160/160	232/234	229/229	159/159	179/179	224/224	143/143	128
E107450	T704	B	EF1	17/1/01	F		EF1 from 2001	B	B	B	160/160	232/234	229/229	159/159	179/179	224/228	145/147	134

Taiko *(Pterodroma magentae)* Genetic Data								Mitochondrial DNA Haplotypes			Microsatellite Genotypes							
Band Number	**Sample Number**	**Status**	**Location**	**Date**	**Sex**		**Comment**	**Cyt *b***	**F1 CR**	**F2 CR**	**Tch6**	**Paequ3**	**Paequ8**	**Paequ13**	**RBG18**	**Tch25**	**RBG29**	**De33 (Sex-linked)**
E108951	T1037 (0.2ml)	C	S19	Apr-07	M													
E108952	T1036 (0.15ml)	C	S18	Apr-07	F													
E108953	T1038 (0.2ml)	C	S35	Apr-07	M													
E108954	T1039 (0.1ml)	C	S40	Apr-07	M													
E108955	T1089 (0.25ml)	C	S29	Apr-07	M													
E108956	T1087 (0.2ml)	C	EF3	Apr-07	M													
E108957	T1088 (0.2ml)	C	EF1	Apr-07	M													
E108958	T1045 (0.2ml)	C	S55	Apr-07	M													
E113511	T1004 (0.4ml)	NB	S76	13/10/03	M		Lights 2003 & 2005, S76 from 2005	A	Y	Y	160/160	228/242	229/229	161/163	179/179	224/224	145/147	128/128
E113512	T1003 (0.3ml)	L	Lights only	13/10/03	F		Lights 2003	A	W	W	160/160	232/236	229/229	159/159	179/179	226/228	147/147	134
E113514	T1020	L	Lights only	29/10/03	M		Lights 2003	A	Y	Y	160/160	228/234	229/229	159/159	179/181	228/228	145/147	128/134
E113515	T1021	L	Lights only	29/10/03	F		Lights 2003	E	E	E	160/160	232/242	229/229	161/161	179/179	224/228	145/145	134
E113516	T1009 (0.15ml)	C	S40	27/4/04	M			C			160/160	232/232	229/229	159/159	179/179	224/228	145/145	134/134
E113517	T1014 (0.2ml)	C	S35	27/4/04	F			A	Y	Y	160/160	234/250	229/229	159/161	179/179	228/228	147/147	128
E113518	T1012 (0.15ml)	C	S19	27/4/04	F						160/160	238/240	229/229	159/163	179/179	224/226	143/147	134
E113519	T1015 (0.15ml)	C	S18	27/4/04	F			G			160/160	234/238	229/229	159/159	179/179	224/224	143/147	134
E113520	T1018 (0.15ml)	C	S55	27/4/04	F						160/160	232/238	229/229	159/159	179/179	224/224	145/147	128
E113521	T1016 (0.2ml)	C	EF3	28/4/04	F						160/160	240/240	229/229	159/159	179/179	228/228	147/147	128
E113522	T1017 (0.2ml)	C	EF1	28/4/04	F			B			160/160	234/236	229/229	159/159	179/179	224/224	145/145	128
E113523	T1019 (0.15ml)	C	S1	4/5/04	M						160/160	232/240	229/229	159/163	179/181	224/226	145/147	128/128
E113524	Feathers only	C	S53	13/5/04	M						160/160	228/240	229/231	159/163	179/179	-	143/145	128/128
E113525	T1025 (0.3ml)	C	S29	11/4/05	F						160/160	232/240	229/229	159/163	179/179	224/228	143/143	134
E113526	T1026 (0.05ml)	C	S1	12/4/05	F						160/160	234/234	229/229	159/159	179/181	224/226	143/147	128
E113527	T1022 (0.1ml)	C	S55	12/4/05	F						160/160	232/238	229/229	159/159	179/179	224/224	147/147	134
E113528	T1050 (little)	C	S40	12/4/05	F						160/160	232/234	229/229	159/159	179/179	224/228	143/143	128
E113529	T1051 (0.2ml)	C	S18	13/4/05	F						160/160	232/238	229/229	159/159	179/179	224/224	143/147	128
E113530	T1052 (0.05ml)	C	S19	13/4/05	M						160/160	238/240	229/229	159/163	179/179	224/224	147/147	128/134
E113531	T1053 (0.2ml)	C	S71	13/4/05	M						160/160	238/250	229/229	159/159	179/181	224/224	143/147	134/134
E113532	T1054 (0.2ml)	C	EF1	14/4/05	M			B			160/160	232/236	229/229	159/159	179/179	224/224	147/149	134/134
E113534	T1040 (0.28ml)	L	Lights only	22/10/05	F		Otawae & Tuku lights 2005	B	B	B	160/160	232/238	229/229	159/163	179/179	228/228	143/147	134
E113535	T1041 (0.34ml)	L	Lights only	22/10/05	F		Lights 2005	A	Y	Y	160/164	240/240	229/229	159/163	179/179	224/224	147/147	128
E113536	T1042 (0.05ml)	L	Lights only	23/10/05	M		Lights 2005	B	B	B	160/160	232/234	229/229	159/159	179/179	224/228	143/145	128/134
E113537	T1043 (0.2ml)	L	Lights only	24/10/05	M		Lights 2005	A	R	R	160/160	236/236	229/229	159/163	179/179	228/228	143/145	128/128
E113538	T1044 (0.18ml)	L	Lights only	25/10/05	F		Lights 2005	D	D	D	160/160	232/242	229/229	159/161	179/179	222/224	143/145	134
E113539	T1046 (0.11ml)	L	Lights only	25/10/05	F		Lights 2005	B	B	B	160/160	234/238	229/229	159/163	179/179	224/226	143/147	128
E113541	T1047 (0.1ml)	L	Lights only	2/11/05	F		Lights 2005	D	D	D	160/160	236/238	229/229	159/159	179/179	224/228	145/147	134
E113542	T1075 (0.15ml)	C	S31	Apr-06	F			G	K	M	160/160	232/236	229/229	159/161	179/179	228/228	143/143	128

Taiko *(Pterodroma magentae)* Genetic Data								Mitochondrial DNA Haplotypes			Microsatellite Genotypes							
Band Number	**Sample Number**	**Status**	**Location**	**Date**	**Sex**		**Comment**	**Cyt *b***	**F1 CR**	**F2 CR**	**Tch6**	**Paequ3**	**Paequ8**	**Paequ13**	**RBG18**	**Tch25**	**RBG29**	**De33 (Sex-linked)**
E113543	T1076 (0.15ml)	C	S29	Apr-06	M				F	F	160/160	230/238	229/229	163/163	179/179	224/228	143/147	134/134
E113544	T1077 (0.15ml)	C	S4	Apr-06	M				S		160/160	238/242	229/229	159/159	179/179	224/228	141/141	128/128
E113545	T1078 (0.2ml)	C	S18	Apr-06	F				K		160/160	234/238	229/229	159/159	179/179	224/224	143/147	134
E113546	T1079 (0.05ml) T1080 (0.1ml)	C	S19	Apr-06	F				V		160/160	238/242	229/229	159/163	179/181	224/226	143/143	134
E113547	T1081 (0.15ml)	C	S53	Apr-06	M				Y		160/160	240/240	229/229	159/161	179/181	224/224	143/147	128/128
E113548	T1082 (0.17ml)	C	S50	Apr-06	M			G	K	M	160/160	232/234	229/229	159/159	179/179	224/224	143/143	134/134
E113549	T1083 (0.15ml)	C	S71	Apr-06	F				V		160/160	236/242	229/229	159/161	179/181	224/224	143/143	128
E113550	T1084 (0.17ml)	C	S55	Apr-06	M				V		160/160	232/238	229/229	159/159	179/179	224/228	145/147	128/128
E113855	T1085 (0.08ml)	C	EF3	Apr-06	F				Y	Y	160/160	230/240	229/229	163/163	179/179	224/228	143/143	134
E113856	T1086 (0.2ml)	C	EF1	Apr-06	M				B	B	160/160	234/236	229/229	159/161	179/179	224/228	145/149	128/134
E127206	T810	B	S53	21/10/01	M		Lights 1982, S53 from 2001	H			-	232/236	-	-	-	-	-	-
E127207	T808	B	EF3	18/10/01	M		Lights 1982, EF3 from 2001	G	K	M	160/160	232/240	229/229	159/159	179/179	224/228	143/147	128/134
E127210	T1005 (0.4 ml)	?	Tuku Burrow	13/10/03	F		Lights 1982 & 2003, tracked to Tuku burrow	A	Y	Y	160/160	240/250	229/231	163/163	179/179	224/224	143/147	128
E127228	T305	NB	S32	29/10/99	M		Lights 1985 & 1999, S32 1999	A	W	W	160/160	232/236	229/229	159/159	179/179	224/224	147/147	128/134
E127229	T56	B	S1	1/10/96	F		Lights 1986, S1 from 1990	G	L	M	160/160	232/234	229/229	159/159	179/181	224/228	143/145	128
E127230	T1010 (0.25ml)	B	S50	7/10/03	F		Lights 1987, S50 from 2003	A	T	Q	160/160	236/238	229/229	159/159	179/179	224/228	143/143	134
E127242	T58 T130 (0.3ml)	B	S18	1/10/96	M		Lights 1992 & 1993, S18 from 1993	A	V	Q	160/160	238/238	229/229	159/159	179/179	224/224	147/147	128/134
E127245 / E192262	T201 (0.2ml)	B	S40	24/4/01	M		S40 from 1999	E	E	E	160/160	232/242	229/229	159/161	179/179	224/224	143/143	128/134
E127246	T606 (0.15ml)	NB	many burrows	1/11/00	M		S38, S62, S41, TR99-17, S40 see Oct 2004 report	A	W	W	160/160	236/250	229/229	159/161	179/179	224/228	143/147	128/128
E127247	T603 (0.15ml)	B	S35	29/10/00	M		S35 from 1999	A	W	W	160/160	240/250	229/229	159/161	179/179	224/228	147/147	128/134
E127248	Feathers only	B	S46	2/12/99	M		S46 from 1999	-	-	-	-	-	-	-	-	-	-	-
E127249	T801	L	Lights only	11/10/01	M		Lights 2001, Otawae lights 2003	A	Z	Z	160/160	228/240	229/229	159/159	179/179	-	143/145	128/134
E127250	T804	NB	EF burrows	14/10/01	M		Lights 2001 & 2005, EF burrows see Oct 2006 report	J	O	O	160/160	232/236	229/229	159/163	179/179	228/228	143/145	128/134
E127251	T59 (0.4ml?) T60 (0.1ml?) A02 (0.4ml?)	L	Lights only	1/10/96	M		Lights 1996	B	B	B	160/160	234/236	229/229	159/161	179/179	228/228	143/145	128/134
E127252	SB6	NB	EF4	23/11/01	M		EF4 2001	A	Y	Y	160/160	230/234	229/229	159/159	179/179	224/228	147/147	134/134

Taiko *(Pterodroma magentae)* Genetic Data								Mitochondrial DNA Haplotypes			Microsatellite Genotypes							
Band Number	**Sample Number**	**Status**	**Location**	**Date**	**Sex**		**Comment**	**Cyt *b***	**F1 CR**	**F2 CR**	**Tch6**	**Paequ3**	**Paequ8**	**Paequ13**	**RBG18**	**Tch25**	**RBG29**	**De33 (Sex-linked)**
E127253	T1006	L	Lights only	13/11/03	F		Otawae lights 2003	I	I	I	160/160	240/250	229/229	159/161	179/179	224/224	141/147	128
E127254	T1007	L	Lights only	13/11/03	M		Otawae lights 2003	A	Y	Y	160/160	232/240	229/229	159/159	179/179	228/228	143/147	134/134
E127255	T1008	L	Lights only	13/11/03	F		Otawae lights 2003	G	K	M	160/164	232/238	229/229	159/163	179/179	224/228	143/149	134
E177254	T53	B	S10	1/10/96	F		S10 from 1990	B	B	B	160/160	238/238	229/231	159/161	179/179	228/228	145/145	134
E177256	T57	B	N1	1/10/96	M		N1 from 1990, Died 14/11/96	J	O	O	160/160	234/238	229/229	159/163	179/179	224/228	143/143	134/134
E177257	T1030	B	S35	1/10/06	F		S35 from 2006	G	K	M	160/160	232/232	229/229	159/159	179/179	224/224	143/143	134
E177258	T1034	B	S50	1/10/06	F		S50 from 2006	G	K	M	160/160	232/232	229/229	159/159	179/179	224/224	143/145	134
E177259	T1035	NB	S78	1/10/06	M		S78 from 2006	E	E	E	160/160	232/242	229/229	159/161	179/179	224/228	143/143	128/134
E177591	T51 T52	B	S10	1/10/96	M		S10 from 1991	A	V	Q	160/164	236/242	229/229	159/161	179/179	228/228	143/145	128/134
E188524	T129 (0.3-0.4ml)	NB	S26	1/10/97	M		Lights 1997, S26	A	Y	Y	160/160	228/242	229/231	159/163	179/179	224/224	143/145	128/134
E192252	T150	C, B	S71	27/4/98	F		S19 chick 1998, S71 from 2004		V	Q	160/160	238/242	229/229	159/161	179/181	224/226	143/143	134
E192253	T151	C	S4	15/5/98	M						160/164	236/240	229/229	159/161	179/179	224/228	141/145	128/128
E192257	TK100 (0.15ml)	C, NB	near S19	29/4/99	M		S19 chick 1999, Lights 2003 tracked near natal burrow				160/160	238/240	229/229	159/163	179/181	224/224	143/143	128/128
E192258	TK101 (0.3ml)	C	S4	4/5/99	F						160/164	238/240	229/229	159/159	179/179	224/226	141/143	128
E192259 / E192260	T705	NB	EF2	17/1/01	M		Otawae lights 2003, EF2 from 2006	H	H	H	160/160	236/236	229/229	159/161	179/179	224/224	143/149	128/134
E192261	T209 (0.25ml)	B	S55	24/4/01	F		S55 from 2001	A	V	Q	160/160	238/238	229/229	159/159	179/179	224/224	147/147	128
E192263	T156 (0.25ml)	C	EF3	25/4/01	M				Y		160/160	240/240	229/229	159/163	179/179	228/228	143/147	128/134
E192264	T203 (0.3ml)	C, NB	near EF2	25/4/01	M		EF1 chick 2001, near EF2 2006				160/160	232/238	229/229	159/161	179/179	224/228	145/145	128/134
E192265	T711 (0.1ml)	C	S48	25/4/01	F				E		160/160	234/242	229/229	159/159	179/181	224/224	145/145	134
E192266	T158 (0.2ml)	C	S55	27/4/01	F						160/160	232/238	229/229	159/159	179/179	224/224	147/147	128
E192267	T710 (0.15ml)	C, L	Lights	28/4/01	F		S40 chick 2001, Lights 2005				160/160	232/234	229/229	159/161	179/179	224/228	143/143	134
E192268	T802 (0.1ml)	B	S1	13/10/01	M		S1 from 2001	A	Y	Y	160/160	234/240	229/229	159/163	179/179	226/228	147/147	128/128
E192269	T803 (0.15ml) SB19 (0.35ml)	B	S4	13/10/01 13/10/02	F		S4 from 2001	A	S	S	160/160	238/240	229/229	159/159	179/179	224/226	141/141	128
E192270	T805	B	S48	17/10/01	F		S48 from 2001	E	E	E	160/160	232/234	229/229	159/161	179/179	224/224	143/145	134
E192271	T806	NB	many burrows	17/10/01	M		near S64, TR99-18, S71 2006	E	E	E	160/160	232/242	229/229	159/161	179/179	224/228	143/143	128/134
E192272	T807 T1031 (0.2ml)	NB	S64	17/10/01 1/10/06	M		Otawae lights 2003, S64 from 2004	A	W	W	160/160	232/250	229/229	159/159	179/179	224/228	141/145	128/134

Taiko *(Pterodroma magentae)* Genetic Data								Mitochondrial DNA Haplotypes			Microsatellite Genotypes							
Band Number	**Sample Number**	**Status**	**Location**	**Date**	**Sex**		**Comment**	**Cyt *b***	**F1 CR**	**F2 CR**	**Tch6**	**Paequ3**	**Paequ8**	**Paequ13**	**RBG18**	**Tch25**	**RBG29**	**De33 (Sex-linked)**
E192273	T811	L	Lights only	24/10/01	M		Lights 2001 landed south Waipurua upper Timihonga catchments, Otawae lights 2003	A	R	R	160/160	234/236	229/229	159/161	179/179	228/228	145/147	128/128
E192274	E192274	B	S29	9/11/01	M		Lights 2001, S29 from 2003	A	Y	Y	160/160	230/240	229/229	163/163	179/179	224/228	143/147	134/134
E192275	E192275	C	EF1	22/4/02	F						160/160	234/236	229/229	159/161	179/179	224/228	147/149	134
E192276	E192276	C	EF3	22/4/02	F				Y		160/160	230/232	229/229	159/159	179/179	224/228	147/147	128
E192277	T159 (0.2 ml)	C, L	Lights	22/4/02	F		S19 chick 2002, Lights 2005				160/160	228/242	229/229	159/163	179/181	224/226	143/143	128
E192278	P2190 (0.3 ml)	C	S18	22/4/02	M			G			160/160	232/238	229/229	159/159	179/179	224/224	143/147	128/134
E192279	E192279 (0.1ml)	C	S40	2/5/02	M			C			160/160	232/234	229/229	159/159	179/179	224/228	143/143	134/134
E192280	E192280 (0.1 ml)	C	S55	3/5/02	M						160/160	232/238	229/229	159/159	179/179	224/228	145/147	128/128
E192283	Feathers only	C	S1	10/5/02	F						-	-	-	-	-	-	-	-
E192284	SB16 (0.45ml)	B	EF1	6/10/02	M		EF1 from 2002	J	P	P	160/160	236/238	229/229	159/161	179/179	224/224	145/149	128/134
E192285	SB17 (0.35ml)	B	S29	8/10/02	F		S29 from 2002	F	F	F	160/160	232/238	229/229	159/163	179/179	224/228	143/143	134
E192286 / E192287	SB18 (0.35ml)	B	EF3	10/10/02	F		EF3 from 2002	A	Y	Y	160/160	230/240	229/229	159/163	179/179	224/228	143/147	134
E192288	T1002 (0.35ml) SB39	C	S18	6/5/03 5/4/03	F			G			160/160	232/238	229/229	159/159	179/179	224/224	143/147	134
E192289	E192289 SB38	C	S19	1/5/03 5/4/03	F						160/160	228/240	229/229	159/163	179/181	224/226	143/147	134
E192290	E192290 SB27	C	S48	1/5/03 5/4/03	M						160/160	232/238	229/229	159/161	179/179	224/224	143/145	128/134
E192291	T1000 (0.3ml) SB40	C	25-14	6/5/03 5/4/03	M				K	M	160/160	232/240	229/229	159/161	179/179	224/224	143/143	128/128
E192292	T1001 (0.3ml) SB28	C	S50	6/5/03 5/4/03	F				T		160/160	238/242	229/229	159/159	179/179	228/228	143/143	134
E192293	P2189 (0.2ml) MO159	C	EF3	30/4/03 6/4/03	F						160/160	230/232	229/229	159/159	179/179	224/224	147/147	128
E192294	E192294 MO151	C	EF1	30/4/03 6/4/03	F						160/160	232/236	229/229	159/161	179/179	224/228	147/149	128
E192295	P2193 (0.2ml) MO160	C	S31	30/4/03 6/4/03	F			G	K		160/160	232/236	229/229	159/161	179/179	224/228	143/143	128
E192296	P2191 (0.15ml)	C	S55	29/4/03	M						160/160	232/238	229/229	159/159	179/179	224/224	147/147	128/134
E192297	E192297	C	S53	1/5/03	M				Y		160/160	240/242	229/231	159/163	179/179	224/224	143/145	128/128
E192298	E192298 (0.2ml)	C	S1	6/5/03	F			G			160/160	232/240	229/229	159/159	179/181	226/228	145/147	128
E192299	T1011 (0.2ml)	B	S31	8/10/03	F		S31 from 2003	G	K	M	160/160	232/238	229/229	159/159	179/179	228/228	143/145	134
E201552	T601 (0.15ml)	B	S31	26/10/00	F		S31 from 2000	G	K	M	160/160	236/238	229/229	159/159	179/179	224/228	143/145	134
E201553	T602 (0.15ml)	NB	S47	27/10/00	M		S47 from 2000	A	V	Q	160/160	238/238	229/229	159/159	179/179	224/226	145/147	134/134
E211552	T1023 (0.2ml)	B	S53	6/10/04	F		S53 from 2003	A	Y	Y	160/160	228/240	229/231	159/163	179/181	224/224	143/147	128

Nāu te rourou, Nāku te rourou, Ka ora ai te iwi

With your contribution and mine the Tāiko can survive